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CONTENTS

INVESTIGATIONS INTO YELLOW-LEAF DISEASE OF PHORMIUM

I. History and Symptomatology. *W. R. Boyce* and *F. J. Newhook* .. 1

II. Translocation Disorders. *K. Strzemienski* 12

III. Anatomical Aspects. *F. J. Newhook* 17

IV. Experimental Induction of Yellow-Leaf Condition in *Phormium tenax* Forst. by the Insect Vector *Oliarus atkinsoni* Myers. (Hem., Cixiidae). *R. A. Cumber* 31

V. Transmission Experiments and Discussion on Control. *Sheila W. Boyce, W. R. Boyce, E. E. Chamberlain, P. R. Fry, R. E. F. Matthews* and *F. J. Newhook* 41

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FIG. 1. Yellow-leaf diseased plant, of '56'. Advanced stage of disease.

FIG. 2. 'SS' left, '301' S_1 right. These plots were planted in 1941; the survival of 'SS' is in marked contrast to the almost complete loss of '301' S_1.

Note FIG. 1 and 2 were photographed in August 1951 before the marked seasonal increase in yellowing had occurred.

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VOL. 34. SEC. A 1953 SUPP. 1.

INVESTIGATIONS INTO YELLOW-LEAF DISEASE OF PHORMIUM

I. HISTORY AND SYMPTOMATOLOGY

By W. R. BOYCE, Botany Division, and F. J. NEWHOOK, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 10 June 1952*)

Summary

Yellow-leaf disease of phormium (*Phormium tenax*) was first observed about 1908. During the following ten years many serious outbreaks of the disease occurred with the result that a number of phormium areas ceased production. The disease which infects both phormium and mountain phormium (*P.colensoi*), occurs throughout the North and South Islands of New Zealand. In the North Island it is a limiting factor in economic production of phormium fibre.

Since 1919 a number of workers have investigated the mycology, bacteriology and physiology of yellow-leaf infected plants but have been unable to demonstrate the cause of the disease. Recently it has been shown that phormium yellow-leaf is caused by a sap-transmissible virus which is spread in the field by the phormium hopper (*Oliarus atkinsoni*).

Phormium yellow-leaf causes phloem necrosis and xylem gummosis leading to an almost complete blockage of translocation. Secondary symptoms are leaf yellowing, root and rhizome rotting, and, finally, death of the plant.

There is evidence that resistance occurs in at least one cultivar.*

INTRODUCTION

A PRELIMINARY note (Boyce *et al.*, 1951.) reported that recent investigations had shown that yellow-leaf disease of phormium (*Phormium tenax*. Forst.) is caused by a virus transmitted by at least one insect vector, *Oliarus atkinsoni* Myers, by mechanical means, and by grafting. There is also circumstantial evidence for seed transmission. The following series of papers report details of the work on which these findings were based.

*The names or numbers used as epithets for cultivars of *P.tenax* are applied to stocks of plants which have been propagated vegetatively from plants selected in the field. Each cultivar is judged from its morphological uniformity to have originated by vegetative propagation from a single plant, but as the exact origin is unknown the term "clone" cannot be strictly applied. Where seedling progenies from these named or numbered cultivars are referred to, this fact is indicated in the text, e.g., S_1 first generation self-bred progeny from plants self-pollinated or open pollinated within the cultivar.

Research carried out by the Department of Scientific and Industrial Research has been centred on the Government-owned Moutoa Estate Phormium Development Area, near Foxton. The commercial development of the estate has been the responsibility in turn of the following Departments:—Public Works, Industries and Commerce, and, since 1949, Agriculture. Material assistance in the investigations has been given by these Departments, in particular by the Department of Agriculture. Valuable help at many times has been provided by New Zealand Woolpack & Textiles Ltd., Foxton.

DISTRIBUTION OF YELLOW-LEAF

Most observations on the occurrence and spread of the disease have coincided with periods of more intense activity in the industry. In a personal communication to the authors, Mr E. W. Sutton, Mill Superintendent New Zealand Woolpack and Textiles Ltd., Foxton, stated that the first outbreak known to the industry was brought to his attention in the year 1908. This occurred in an area of phormium owned by L. Seifert in the Rangitane district near the present position of Aker's toll bridge. The attack was severe and destroyed much phormium in a localized area. The next outbreaks to be observed occurred in 1914-16 in the lower reaches of the Manawatu River at Tokomaru and Makerua, (L. Cockayne, 1920). By 1920 the disease was widespread in the Manawatu and had been reported in areas being milled at Waikanae and Plimmerton. In the following years it made its appearance further down the Manawatu River in areas such as Whitanui and was reported from other places in the North Island. As diseased areas became unproductive, they were in almost all cases cleared and converted to farm land; but many other factors played a part, especially during periods of depression in the industry, and this makes it impossible to assess independently, the part played by yellow-leaf in this trend to clearance.

By 1940, the Moutoa area alone in the Manawatu remained fairly free of the disease, but since 1944 a rapid deterioration has taken place in that area also.

More recent observations (since 1945) have shown that the disease is widespread throughout the North Island, not only in milling areas, but also in undeveloped swamps, in small stands and in isolated bushes in many situations. The disease is present in milling areas at Kaingaroa, (near Awanui), Dargaville and Kerepehi, and on the Ruamahanga River, near Martinborough and Featherston, as well as at Moutoa. At Dargaville and Featherston the disease has scarcely affected production, whereas at Moutoa it has reduced considerably the production from induced areas and plantations.

With regard to the distribution in the South Island, L. Cockayne (1920) stated, "throughout the South Island with hardly an exception there is more or less yellow-leaf. Nor are the diseased plants confined to milling areas or even to swamps. On the contrary they occur amongst plants of dry stations at all altitudes up to the altitudinal limits of the plants; indeed there is possibly no situation where the flax plants can live secure". During more recent surveys in the South Island the disease has been noted on the West coast at Karamea, Westport, Barrytown, Runanga, and near the Karengarua River. In Southland it has been seen at Riverton, Gorge Road, and Wyndham; and in Canterbury at Waikuku near Christchurch. No epiphytotic is known to have developed in the South Island up to the present time.

Although recorded observations on the occurrence of yellow-leaf in mountain phormium (*P. colensoi* Hook.f.) have been confined to relatively few localities during the present investigation the disease has been noticed in the field occasionally. *P.colensoi* planted at Moutoa has proved to be highly susceptible. F_1 plants of several crosses between *P.tenax* and *P.colensoi* have also been highly susceptible.

History of Research

In the New Zealand Department of Agriculture Annual Report (1909), Kirk and A. H. Cockayne in an account of the pests and diseases of New Zealand phormium make no reference to yellow-leaf. A disease causing leaf yellowing of phormium seedlings, then thought to be due to *Fusarium* spp., is however mentioned. This might possibly have been yellow-leaf, followed by secondary invasions of *Fusarium* or *Glomerella* spp., as noted frequently in recent investigations (Newhook, 1949). Formal recorded research began in 1916, according to the same departmental reports.

Since 1919, many workers have contributed to research on yellow-leaf disease, with a view to finding the cause and developing economic methods of control. This work has been marked by inability to associate the disease with a consistently occurring pathogen or to demonstrate a physiological cause. In the absence of a demonstrable cause, the selection and propagation of apparently resistant varieties has been advanced as the most satisfactory method of control. Observations over many years made it clear that epiphytotics of the disease followed extensive draining and protection from flooding of phormium stands, but whether the changed environment was favouring the spread of a disease or simply causing physiological deterioration of the plants was still undecided until recently. It now appears certain that the altered conditions influenced the breeding of the insect vector *Oliarus atkinsoni*.

Most workers have agreed that leaf yellowing and wilting are secondary symptoms following the loss of roots. Recent work (Newhook, 1953) has shown that degeneration of the rhizome vascular bundles precedes root and leaf symptoms.

L. Cockayne (1920) carried out observations to determine what conditions of soil or climate favoured or hindered the disease. Initial observations in the Manawatu areas led him to believe that the disease was physiological in nature, caused by unfavourable water table conditions. After further observations in both islands he altered his opinion, and decided that the disease was probably pathogenic, being caused by either a fungus or bacterium. The distribution of the disease in many different habitats throughout the South Island and the very rapid increase in the disease in the Manawatu in 1918-1919, were the main reasons for his changed opinion.

Waters and Atkinson (1922) investigated fungi and bacteria associated with the roots of diseased and healthy plants from various localities in the North and South Islands. A fungus, *Ramularia* [*Cylindrocarpon*] sp., was suspected as a possible pathogen, but these workers later concluded that "in the light of our present knowledge it cannot be assumed that a micro-organism is certainly the cause." Further experiments were carried out to ascertain whether a pathogen was carried in the soil surrounding diseased plants, in diseased roots, or in the seed, and further tests for the pathogenicity of *Ramularia* sp., were made. No conclusive results were reported from these experiments.

By 1930 many induced phormium areas had been lost to production. Attempts had been made to replace them with plantations, which were stocked in some cases with plants which had undergone selection for quality, yield, and apparent disease resistance. Attention was focussed on the need for further research into methods of preserving the remaining indigenous areas, and of ensuring the success of plantation establishment.

Cunningham (1930) drew up a research plan assuming that yellow-leaf could be caused by insects, fungi, bacteria, a virus, or non-pathogenic and purely physiological factors. He stressed the importance of an investigation of the virus hypothesis by analogy with other "yellows" diseases; the importance of a survey of the insect fauna of phormium areas for possible insect vectors was noted. The scheme was not implemented in full.

From 1930 to 1938 yellow-leaf research was carried out at Massey College under the direction of Yeates, along the following lines:

(1) *Selection of, and breeding from, resistant plants.* Yeates (1931) reported that one cultivar (presumably 'SS') which had been under observation by Mr. A. Seifert, for many years was resistant to if not immune from the disease. Later (1935), this view was modified, in view of the fact that 'SS' contracted the disease at the Palmerston North area. Another cultivar, '22', was regarded as showing a high degree of resistence from observations over a period of seven years. 'SS' was inbred for three generations, and crossed with other commercially valuable plants. Later experience at Moutoa has confirmed the original opinion on 'SS'; under a wide range of field conditions it is apparently resistant but not immune, while '22' has not shown marked resistance.

(2) *Manurial treatment for possible soil deficiencies.* Manurial trials (Yeates, 1935) with nitrogen, phosphorus and potash treatments did not reduce the incidence of the disease, but showed that plants under good growth conditions (judged on the growth of adjacent non-diseased plants) contracted the disease.

(3) *Investigation of fungi and bacteria.* Meadows (1929) carried out field examinations of diseased plants, microscopic examination of diseased and healthy roots, and isolations of fungi and bacteria. Field examinations confirmed the association of a high percentage of decayng roots with leaf yellowing. Plants in dry sandy situations and in swamps, showed equivalent degrees of yellowing. Microscopic examinations showed that hyphae were frequently present in diseased root tissue; these fungi were not identified in field examinations and could well have been either mycorrhizal fungi or secondary saprophytes.

(4) *Virus investigations.* No records are available of virus transmission work carried out. In 1934, the presence of an insect larva (*Porina* sp.) in association with diseased plants was investigated, but no proof of connection with the disease was obtained.

Yeates, Dixon and Taylor (Taylor, 1937) carried out a survey of phormium areas in the North Island. Yellow-leaf was referred to as a general chlorotic condition of the plant and different types were distinguished; on dry infertile soils of the National Park area, yellowing was noted which, it was thought, could have been rectified by the application of fertilizers. Another type of yellowing, accompanied in some cases by rotting of the rhizome, occurred on such a wide variety of soils that the

evidence was against its being a deficiency disease. The worst cases of yellowing occurred on rather infertile soils with a high water table, but it also occurred in odd plants on very fertile and apparently moderately well drained soils, "for no very obvious reason". The good growth of phormium and the absence of yellow-leaf on old Maori plantations was noted. More recent surveys have shown that in at least one place, Maungatapu Pa, Tauranga, yellow-leaf has subsequently appeared in serious proportions in a Maori plantation.

In 1934, Yeates established a nursery for the propagation of selected cultivars of phormium on the Moutoa Estate (Easton Nursery). Yellow-leaf was first noticed in this area in 1937, and in 1938 Chamberlain began mycological investigations of the diseased plants. This work had to be abandoned in 1939, and was not resumed until 1944.

Since 1944, investigations have been centred at the Botany Division Research Station on the Moutoa Estate (where work by Botany, Entomology and Plant Diseases Divisions and by Soil Bureau has been carried out), at Plant Diseases Division, Auckland, and at Soil Bureau Wellington. These are summarised below.

(1) Records were taken over several seasons of the incidence of disease in plantations and the course of disease in individual plants. The pattern of new infections was consistent with spread by air-borne or by insect-borne infection (Newhook, 1951). The incidence and distribution of disease in swamp areas was noted during routine botanical surveys, and the relationship of this distribution to soil types on the Moutoa Estate (mapped by Soil Bureau in 1940), and to water table conditions was checked. Yellow-leaf was found on all soil types but was more frequent on the higher contours near the river bank on silt and sandy silt loams. (Boyce and Roberts, 1949; Roberts, 1950).

(2) Transplanting trials were established in which diseased and healthy fans were planted together, and in which healthy fans were replanted in the positions formerly occupied by diseased bushes. No transmissions were obtained.

(3) Trials for nutritional deficiencies were established using indicator plants. The results of these led to the application, at varying rates, of boron, of other minor elements, and of phosphate, to trial plots of phormium and indicator plants. No response was obtained. Pot trials in conjunction with this work were carried out at Plant Diseases Division, Auckland, (Atkinson, unpublished data) and at Soil Bureau, Wellington (Wright and Gridley, unpublished data). Tissue analyses for boron content were also carried out at Soil Bureau, and spectrographic analyses at Dominion Laboratory. No indications were obtained that yellow-leaf was a nutritional disease.

(4) Mycological examinations of diseased and healthy plants were resumed (Newhook, 1951).

(a) Periodical isolations were made from roots and rhizomes using several media; little consistency was obtained in over 3,000 isolations. Species of *Cylindrocarpon*, *Trichoderma*, *Fusarium* and *Cadophora*, and various bacteria occurred most frequently, but in many examinations of root and rhizome rot culturable or visible organisms were absent. The flora of dead tissue of diseased and healthy plants was similar.

(b) None of the fungi tested produced yellow-leaf symptoms.

(c) No recovery was obtained by trimming dead tissue from diseased fans, surface sterilizing and replanting.

(d) No organism has been isolated from or seen in non-necrotic rhizome, or leaf tissue, or in the vascular bundles of affected plants. Mycorrhiza have been present wherever examinations for their occurrence have been made.

(5) Investigations on the effect of yellow-leaf on translocation have been carried out by Strzemienski (1953) using dyes and radioactive phosphate, and Newhook (1953) who related the observed translocation upset to anatomical changes.

(6) In 1947, examinations at Moutoa of the soil fauna associated with the root systems of plants in diseased plantations revealed the presence of dense populations of cicada nymphs. Since Cunningham (1930) had stressed the need to test possible virus vectors, and because there were many similarities between yellow-leaf and some more recently described viruses, such as phloem necrosis of the American elm, cicada nymph transmission tests were set up with phormium and other test plants. These gave negative results other than a stunting of growth of treated plants. (S. W. Boyce and W. R. Boyce, 1948). The need for a complete entomological investigation was re-emphasised. This was undertaken by Cumber in 1949, who obtained transmission with the plant hopper *Oliarus atkinsoni*. (Boyce *et al.*, 1951; Cumber, 1953.)

(7) At Plant Diseases Division, Auckland, attempts were made to obtain virus transmissions by means of grafting, mechanical inoculation and insect feeding. Transmissions were obtained by mechanical inoculation and with *O.atkinsoni*. (Boyce *et al.*, 1951) and a few grafts gave transmission after a long incubation period.

(8) Selection of, and breeding from, resistant plants of phormium has continued. The following stocks of plants are now available for testing for resistance by inoculation with the virus: inbred seedling progenies of *P.tenax* 'SS', and two other *P.tenax* selections, '311' and 'Tihore', which have shown indications of resistance to the disease in the field; several F2 families from a *P.tenax* 'Ngaro' x *P.tenax* 'SS' cross. (In crosses between 'SS' and susceptible plants, field evidence has shown that the F_1 generations are generally susceptible.) In addition, several other F_1 progenies, including *P.tenax* 'SS' x *P.tenax* 'Tihore', are approaching maturity for the production of F_2 generations for selection purposes.

SYMPTOMATOLOGY

Yellow-leaf disease is characterised by abnormal yellowing of leaves, stunting of growth and premature flowering; discolouration of vascular bundles in the rhizome, death of lateral and main roots, rhizome rotting and collapse of the leaves; and the invasion of all parts by secondary organisms which may hasten the death of the plant.

From the appearance of the first outwardly visible symptoms until the final collapse, the course of the disease may take from a few months to several years. However, as a phormium plant takes from five to seven years to mature for the first cut, and approximately five years for succeeding cuts, both the slow and the rapid onset of the disease cause a complete loss of leaf for harvest.

Leaf symptoms

A normal healthy fan has about eight living leaves attached, with one, sometimes two, of the oldest leaves gradually yellowing, dying back and falling; this yellowing is seldom obvious, nor is there a conspicuous accumulation of recently dead leaves about the base of the fan, except in the case of a flowering fan, where the leaves die back completely after the inflorescence has matured.

A diseased fan usually has more than two leaves per fan conspicuously yellowed and dying at any one time. In some cases with a very rapid onset of disease leaves roll from the margins into the keel while still green, wilt, redden at the base (particularly in certain varieties), turn brown and collapse. (Figs. 1 and 4.) While there is no consistent difference between diseased and healthy bushes in the nature of yellowing other than in the number of leaves affected, incipient stages of yellow-leaf can often be detected by a lighter colouration of the leaves, particularly at the apices.

In a healthy fan, a leaf has reached its maximum height when it has been displaced to the third or fourth position from the centre. Stunting is first apparent in a diseased fan when the third and fourth leaves cease growth before they attain the height of older leaves. With progress of

Fig. 3. Stages of disease from onset (right background) to death of plant, in a plantation of 'Muka'. Moutoa 1946.

Fig. 4. Rapid form of attack. Leaf rolling (without obvious yellowing) in 'Paretaniwha'.

Fig. 5. Death of lateral roots preceding collapse of main root.

Fig. 6. Flattened, tapered, diseased roots with crowded laterals.

the disease there is a marked diminution of the ultimate length of successively produced leaves; this is often accompanied by a characteristic bluntening of the normally acute leaf apex.

Fungi (*Glomerella* spp. and *Fusarium* spp.) and soft rot bacteria often invade the young central leaves of plants in an advanced stage of yellow-leaf.

Root symptoms

Death of lateral and main roots is a normal feature of healthy plants in localities free from yellow-leaf. Roots may be annual, or more frequently they may persist for several years. On diseased plants, however, the amount of root death is very considerably increased. Lateral roots die first (Fig. 5) and eventually the main root collapses, cortical breakdown developing independently of the location of the dead laterals. Dead and living roots of the same or adjacent plants frequently intermingle and new roots often grow down inside the epidermal cylinders of decayed roots. New root production on diseased fans may be quite vigorous during the winter and spring, but the roots are frequently abortive; instead of emerging cleanly through the leaf bases, they may cease growth almost immediately or girdle the stem under the sheathing bases, becoming flattened, sharply tapered, and bearing crowded, often fused, lateral roots. (Fig. 6.)

Rhizome symptoms

Phloem necrosis and xylem gummosis of rhizome vascular bundles, the first detectable symptoms of the disease, have been described by Newhook (1953); the affected bundles vary from a very pale straw colour to deep orange-red. (Fig. 7.)

In the later stages of the disease, the rhizome tissues collapse (Fig. 8); as the rot spreads upwards towards the apex of the rhizome, parenchyma sometimes becomes first translucent and then brown.

Secondary organisms are usually absent from the margins of the rot but further back they cause rapid disintegration of the rhizome, thus separating individual fans of a bush (Fig. 9). On healthy bushes on the other hand, as the older fans flower and die back, their rhizomes remain to form a persistent branching rhizome system.

Progress of disease in the plant

This varies from cases where all fans of a bush may be affected at one time, to cases where the disease takes three or more years to progress from one or two fans on one side to affect the whole bush. Cases have been observed where the progress of the disease seems to be arrested, part of a bush continuing normal growth for some years.

Seasonal variation

There is a well marked seasonal alteration in the appearance of diseased bushes. Yellowing coincides with the period of active growth; in mature plants it appears in August, increases to a maximum from December to February, and from March onwards becomes much less conspicuous as the older yellowed leaves fall, leaving younger, greener leaves on the plant.

Under casual observation, this seasonal variation has often been confused with recovery. It is most marked with bushes in the early stages

of disease; in later stages conspicuous yellowing persists through the winter months. With stands also, the presence of few slightly diseased plants obvious from August to February, may not be noticeable from March to July. However, as the number of diseased bushes and the severity of disease increases, stands retain their yellowed appearance through the winter months.

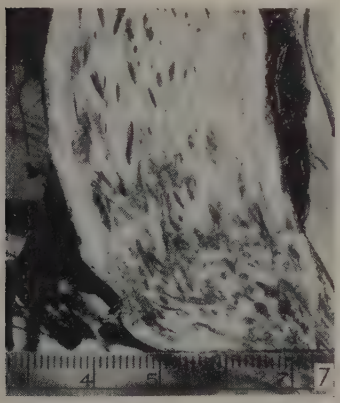

Fig. 7. Red vascular bundles in diseased rhizome.

Fig. 8. Advanced stage of rhizome collapse. Red vascular bundles in remaining rhizome tissue.

Fig. 9. Fans separated by rhizome rot. Roots mainly dead.

Recovery

No case of recovery of individual marked or transplanted diseased fans has been observed. "Recoveries" reported by Cockayne (1920) from observations on marked bushes over a 14-month period, and later cases of apparent recovery were possible examples of seasonal fluctuation in yellowing, arrested progress of disease, or yellowing by causes other than yellow-leaf, e.g., prolonged high water table or drought conditions on perched plants, rather than cases of genuine recovery of diseased fans.

Apparently healthy shoots have occasionally been observed to arise from buds on diseased fans. There is little information available concerning

the movement of virus within the plant, but it appears that at times infection may not become fully systemic in a rhizome or a branched rhizome system. This may explain cases of apparent recovery after death of the original infected fan or fans.

Observations on possible recovery and progress of the disease are difficult in the field as most plants under observation are liable to multiple infections in successive years during the vector flight season.

Period before expression of symptoms

The period between infection and the expression of outward symptoms varies with the age of the plant: young seedlings may show symptoms within a few weeks of infection, but in older plants this period may be extended to 1-3 years.

Resistance

One cultivar, 'SS', has, over a period of fourteen years at Moutoa, shown a high degree of resistance or tolerance to the disease, under a wide range of field conditions. (Fig. 2.) This confirms the observation of Seifert (Yeates, 1931) who propagated this plant more than twenty years ago. The character is not inherited as a dominant in crosses, and its recessive nature should be an advantage in line breeding.

Two other cultivars, '311' and 'Tihore', have shown indications of resistance, but, as only small numbers of these plants have been under observation, further propagation and breeding would be necessary to test their resistance more fully.

Susceptibility of species

Phormium colensoi appears to be at least as susceptible as *P.tenax* under comparable conditions and some *P.tenax* '301' ♀ x *P.colensoi* ♂ F_1 lines have been even more susceptible than the very susceptible *P.tenax* '301'.

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References

Boyce, S. W., Boyce, W. R., Chamberlain, E. E., Cumber, R. A., Fry, P. R., Matthews, R. E. F., Newhook, F. J., and Strzemienski, K. 1951: Preliminary Note on Yellow-Leaf Disease of Phormium. *N. Z. J. Sci. Tech.* A33: 76-77.

Cockayne, L. 1920: Yellow-Leaf Disease in Phormium Tenax. *N. Z. J. Sci. Tech. 3*: 190-6.

—— and Cockayne, A. H. 1919: Yellow-Leaf Disease of Phormium Tenax. *N. Z. J. Sci. Tech. 2*: 256.

Newhook, F. J. 1953: Investigations into Yellow-Leaf Disease of Phormium. III: Anatomical Aspects. *N. Z. J. Sci. Tech. A34*: 17-30.

N. Z. Dept. Agric. 1909: The Pests and Diseases of New Zealand Phormium. *N. Z. Dept. Agric. 17th. Ann. Report. pp. 286-9.*

Strzemienski, K. 1953: Investigations into Yellow-Leaf Disease of Phormium. II: Translocation Disorders. *N. Z. J. Sci. Tech. A34*: 12-16.

Waters, R., and Atkinson, E. H. 1922 : Yellow-Leaf Disease in *Phormium Tenax*: Preliminary report on a current investigation. *N. Z. J. Agric. 24*: 27-32.

Unpublished Reports Not Available For Distribution.

Boyce, S. W., and Boyce, W. R. 1948: Investigation of the Possible Association of Insects with Yellow-Leaf Disease of Phormium. *Bot. Div., Dept. Sci. Ind. Res. Rep.* (Bot. Div. files.)

Boyce, W. R., and Roberts, M. E. 1949: Report on Phormium Surveys. Moutoa Estate. P. 4. *Bot. Div., Dept. Sci. Ind. Res., Rep.* (Bot. Div. files.)

Cunningham, G. H. 1930: Biological Investigations into the Cause of Yellow-Leaf Disease of *Phormium Tenax. Plant Res. Sta., Dept. Agric. Rep. to Flax Res. Comm.* (Plant Dis. Div. files.)

Meadows, L. F. 1929: Summary of Work Done to Date on Yellow-Leaf in Flax. *Massey Coll. Rep. to Dept. Sci. Ind. Res.* (Plant Dis. Div. and Head Office, Dept. Sci. Ind. Res. files.)

Newhook, F. J. 1949: Phormium Yellow-Leaf. Progress Report, October, 1949. *Plant Dis. Div., Dept. Sci. Ind. Res. Rep.* (Plant Dis. Div. files.)

—— 1951: Assessment of Present Position Regarding Yellow-Leaf of Phormium. *Plant Dis. Div. Dept. Sci. Ind. Res. Rep.* (Plant Dis. Div. files.)

Roberts, M. E. 1950: Distribution of Yellow-Leaf in Induced Swamp Areas on Moutoa Estate. *Bot. Div., Dept. Sci. Ind. Res. Rep. for Phormium Res. Comm. Meeting* 1/6/50. (Bot. Div. files.)

Taylor, N. H. 1937: Some Observations on Soil Conditions and Flax Growth in Manawatu and Bay of Plenty Districts. *N. Z. Soil Surv. Rep. 23/11/37.* (Bot. Div. files.)

Yeates, J. S. 1930: Yellow-Leaf Investigation. *Massey Agric. Coll. Rep.* (Bot. Div. files.)

—— 1931: Yellow-Leaf Disease. Report 1/4/31. (Bot. Div. files.)

—— 1935: Report to Flax Research Committee, 13th March 1935. *Massey Agric. Coll. Rep.* (Bot. Div. files.)

INVESTIGATIONS INTO YELLOW-LEAF DISEASE OF PHORMIUM

II. TRANSLOCATION DISORDERS

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Summary

Dye and radioactive phosphate solutions were applied to *Phormium* roots whose distal parts were cut off, and the movement of the introduced substances observed. Gross and consistent differences between healthy and diseased plants were found. The rhizome of affected plants was considered the most likely location of the blockage preventing or restricting the upward movement of solutes. Some observations were also made on the pattern of vascular connections between roots and leaves in the healthy plant and on differences in the movement of phosphate and dye.

Introduction

Before the nature of yellow-leaf disease was clarified, the possibility had been considered that impaired absorption of nutrients by the roots might be the main and initial factor in the development of the outward symptoms (Boyce and Newhook, 1953). Accepting a suggestion from the Phormium Research Station, the writer undertook to investigate this question, using the radio-active isotope of phosphorus as a tracer. Dyes were applied in preparatory experiments for outlining the vascular root-to-leaf connections in the normal plant. This was suggested by the work of Roach (1939) who used dyes extensively for similar purposes. Results of their application to affected plants suggested that differences in translocation rather than absorption should be studied. Experiments with dyes as well as with radioactive phosphate showed that a serious blockage of translocation does exist in plants affected by yellow-leaf disease.

Preliminary Experiments with Dyes

In January, 1949, radioactive phosphate solution was applied to intact roots of presumably healthy phormium plants. One root only in each plant was treated. Leaf samples collected subsequently at various dates were sent to this laboratory and the radioactivity measured. Since results were quite erratic, these experiments were not continued, and dyes, being much easier to manage, were introduced in the later, but still preparatory, experiments commenced in February, 1950.

In holes dug in the neighbourhood of phormium plants in the field, one uninjured root from each plant was selected and cut across, if possible under water. The stump was immersed in the dye solution contained in a short test tube or U-shaped class tube, for various periods of time ranging up to several days. Acid fuchsin (BDH Standard Stain) was very useful because of its quick ascent and excellent colour contrast in the leaf. Leaf injury by the dye was observed only once, when using a 1 per cent solution. Thereafter 0.1 to 0.2 per cent solutions were used. Light green SF (BDH Standard Stain) in 0.1 to 0.5

per cent solutions gave a much better contrast in the orange-coloured rhizome and was used for this reason. Later this dye was not available in sufficient quantity and a suitable substitute was found in naphthalene green GS (ICI). Disulphine blue VNS (ICI), a dye akin to patent blue, applied in 0.1 per cent solutions, also proved useful for similar purposes.

Most of these experiments were made with plants from a miscellaneous collection of selected varieties, progenies and hybrid progenies, transplanted from Palmerston North to Moutoa in 1940.

Experiments with acid fuchsin applied to single roots showed that apparently the vascular connections between root and leaf of Phormium are extremely varied. Sometimes only one leaf showed red stripes, sometimes several leaves of one fan, while sometimes leaves in several fans of the same plant responded. The dye appeared as a rule in stripes consisting of neighbouring parallel strands. There may be several such stripes in a leaf, or only one. The same side of each leaf in a particular fan may show the red striation, or only one half of the fan, or else the distribution may not show any apparent order. Sections of leaves showed under the microscope that the vessel contents were red, but not necessarily all vessels of a strand carried the dye. Often, out of say three vessels in a bundle only one would have red contents.

Warm and dry weather prevailed over most of the late summer of 1950. The soil moisture was low and the transpiration rate must have been high. Under these conditions the ascent of the coloured liquid was fairly fast. The highest rate recorded was of the order of 0.5 m. per hour, in an experiment carried out between 1 and 5 p.m.

Some experiments with acid fuchsin and with light green were made also with diseased plants using the same technique as with normal ones. In several such experiments, the dye applied to a single root did not appear in the leaves. In the rhizomes of healthy fans, the upward movement of the dye into the leaves could be easily traced, especially if light green was used. In rhizomes of yellow-leaf fans the dye would, as a rule, travel into the peripheral layers of the rhizome and would often move from there into a neighbouring root and perhaps downwards a little (both things may happen in a healthy fan)—but with very little upward movement, and without reaching the leaf bases.

Experiment with Radioactive Phosphate

The work with dyes was originally designed to supply information to be used as a guide in conducting experiments with radioactive phosphate. When it was found that dye applied to single cut roots of fans with yellow-leaf did not appear in the leaves it became obvious that there is some difference in translocation between healthy and diseased plants. If in an investigation of the absorbing ability of roots, radioactive phosphate had to be used conveniently and effectively, its concentration in some place reasonably distant from the place of its application had to be measured. Thus two unknowns would be involved at one time: absorption *and* translocation. It was therefore decided at this stage to concentrate first on the translocation problem alone—since this could be studied quite independently of differences in absorbing ability of the roots—and to postpone enquiry into the absorption problem.

The vascular connections between roots and leaves of this species appeared unpredictable in the light of experiments described in the previous section. Newhook (1953) in a later investigation established

some systematic pattern for these connections, and showed that some predictions can be made about them if the exact position of the particular root on the rhizome is known. This position is not known, however, if single roots are being operated upon in an otherwise intact and *in situ* plant. This knowledge brought to light by Newhook was not available at the time these experiments were made. But even if it had been, it would not have changed the fact that working with single roots would be inadvisable in a conclusive experiment on translocation differences; to minimize the influence of chance, an unmanageable number of pairs, each composed of a healthy and an affected fan, would be needed to arrive at even the roughest quantitative estimate.

The experiment described below was designed in accordance with the reasoning outlined in the two preceding paragraphs.

For the experiment with radioactive phosphate one healthy plant and one with unmistakable yellow-leaf disease were selected from a field of the Ngaro variety. The best fan of the diseased plant was separated with its part of the rhizome, and as many roots as looked reasonably healthy—eleven in number—were cut and the stumps dipped in a radioactive solution containing 250 p.p.m. KH_2PO_4 with approximately 270 microcuries of P^{32} per litre. At the same time a healthy fan of comparable size, taken from the healthy plant, was similarly isolated and its cut roots—12 in number—dipped in the same solution on 11 March, 1950. The plants were placed in the glasshouse and tied to the bench in an upright position. The radioactive solution was contained in test tubes, a separate test tube for each root. The activities were followed by repeated measurements with a portable battery-operated Geiger-Muller counter, designed and built by the Dominion Physical Laboratory. Measurements were made at about 70 cm. above leaf base, with probe close to the leaf.

First detectable radioactivity appeared about 40 hours after the application of the radioactive solution, and after 54 hours from the start, the following radioactivities were recorded in counts per minute uncorrected for background (which was about 20 counts per minute):

Healthy fan: 145, 50, 64, 100, 91, given in the order of increasing leaf age;

Yellow-leaf fan: 142, for the youngest leaf, all other leaves at background level.

On 14 March, when radioactivity measurements on leaves did not show any further changes, the set-up was dismantled. At the Soil Bureau Laboratory, cores of 10-mm. diameter were cut out at root insertions with a cork borer, so that they contained a short piece of the root and the adjacent rhizome tissues. Cut down to 5-mm. length, measured from the surface of the rhizome after removing the outer dark dead-tissue layer, they were mounted in snugly fitting glass tubes of 4.5 cm. length and by means of a rubber stopper fitted into the hole of a brass plate. The internal end of the core faced the counter tube. This gave a well standardized "geometry". These radioactivity measurements, corrected for background and difference in time, gave the following figures in counts per minute:

Healthy fan: 404, 788, 808, 830, 1134, *1184*, 1266, 1470, 1472, 1646, 1746;

Yellow-leaf fan: 13, 51, 58, 61, 62, *68*, 72, 121, 146, 347, 471. (Medians shown in italics)

FURTHER EXPERIMENTS WITH DYES

As a confirmatory test some further experiments with dyes were made in April 1950. The whole rhizome was placed in a dye so that the dye was available to all root stumps of each fan. Cross sections showed coloured vascular bundles in rhizomes of both healthy and diseased plants, but there was noticeably less colour in the diseased plant.

Leaves that turned entirely yellow because of advanced disease, when put in a dye, developed stained bundles if the leaves were cut above the "butt". In such circumstances ascent of dye even into completely dry leaf tips was observed. Leaves from healthy or diseased plants if cut low at the base did not take up the dye, probably because of heavy gumming. This occurrence made a direct check on leaf bases in diseased plants ineffective.

DISCUSSION

Physiological experiments such as those described in this paper are often criticized on the ground that a wound opening the vascular system distorts the distribution of pressures that existed in the intact plant, and in consequence a false picture of the transporting channels may be obtained. The validity of this objection cannot be entirely denied. Yet if under the conditions of a high transpiration rate and a rather low moisture content of the soil, only one root is abundantly supplied with water, it should be expected that most xylem connections of the treated root would be used to the full. It does not therefore appear probable that the pattern of xylem connections between roots and leaves of healthy plants, as shown by dyes, could be greatly distorted. This question, however, though it influenced the planning of the experiments, is of only limited importance, as the main problem was to know how the transport of water and solutes in phormium plants affected by yellow-leaf disease, differs from that in normal ones.

Various observations made during this investigation seem to suggest that phosphate moved through some different channels from the dyes. The observable upward movement of phosphate appeared to be always very slow. In one case at least, when a fuchsin-containing radioactive phosphate solution had been applied to a cut root, a young leaf of the treated plant showed higher activity in the half containing less fuchsin.

Without going into a discussion of the still highly controversial question of the translocation paths of mineral nutrients (for reviews of this controversy cf. e.g., Miller, 1938; and Curtis and Clark, 1950), it can be said that it would be astonishing if phosphate ions and the dyes commonly used in such experiments remained together for any length of time. The generally useful ones are acid dyes; they diffuse out of the xylem very little, if at all, and have no ability to penetrate into living cells. The phosphate ion, on the other hand, does diffuse easily, and being an essential constituent of all living matter can enter into living plasma. Indeed, movement of phosphate not only through the sieve tubes but even through the parenchyma has been reported as the means of translocation of this ion (Colwell, 1942). As Newhook (1952) has later shown, the blockage of translocation in the rhizome of phormium attacked by the yellow-leaf disease results from a phloem necrosis and gummosis of the xylem. This would explain why, in these experiments, similar results were obtained with dyes and phosphate, even though they may move through different channels.

The results obtained in this investigation suggested that:

(1) neither radioactive phosphate nor dyes were translocated as freely in yellow-leaf fans as they were in healthy ones. While all green

leaves of the normal fan showed radioactivity, only the youngest leaf of the diseased fan did, though this fan still had several other green leaves;

(2) roots in yellow-leaf fans were capable of translocating either dye or phosphate to the rhizome. It was not in the root, therefore, that the obstacle resided.

The reasons for assuming that the blockage does not reside in the roots were as follows: In most experiments with dyes and single roots the stained trace extended from the root into some closely adjacent parts of the rhizome where it ended. This was so whether the duration of the experiment was half a day or several days. The impression was therefore that the dye could move up to some point in the rhizome where it would stop whatever length of time would be allowed for further ascent. An apparent argument against the assumption in (2) above could be seen in the very much lower radioactivities of the excised root insertions reported on page 14. The root stumps of these experimental plants were, however, carefully examined by another worker*, and in neither fan the apparent state of health of the root seemed related to the radioactivity later recorded. The differences in radioactivity between the cores of the healthy and affected fans could be easily explained in another way. If the ascending translocation stream in the vessels is slowed down by some obstacle placed higher up, then in a given time less liquid and therefore less phosphate would pass through the root; consequently, less would be absorbed and accumulated in the tissues of the insertion region.

On the other hand experiments with dyes applied to cut leaves did not suggest that the main obstacle resides in the leaves. This, together with the evidence previously discussed, suggested that the blockage is caused by some disorder located in the rhizome.

Conclusions

(1) In Phormium plants affected by yellow-leaf disease a blockage of translocation has been demonstrated by the impairment of upward movement of dye and radioactive phosphate solutions.

(2) The rhizome was shown to be the most probable seat of this blockage.

Acknowledgments

The author wishes to express his obligation to Mr. and Mrs. W. R. Boyce, Dr. F. J. Newhook, and Mr. J. A. Swindlehurst, for hospitality and assistance at Moutoa, and to Mr. G. J. Fergusson of the Dominion Physical Laboratory for development of special apparatus.

References

Boyce, W. R. and Newhook, F. J. (1953): Investigations into Yellow-leaf Disease of Phormium. I. History and Symptomatology. *N.Z. J. Sci. Tech. A34* (*Supp. 1*): 1-11.

Colwell, R. N. (1942): The Use of Radioactive Phosphorus in Translocation Studies. *Amer. J. Bot. 29*: 798-807.

Curtis, O. F. and Clark, D. G. (1950): An Introduction to Plant Physiology pp. 240-468. McGraw-Hill, New York. 752 pp.

Miller, E. C. (1938): Plant Physiology. 2nd ed. pp. 876-80. McGraw- Hill, New York. 1201 pp.

Newhook, F. J. (1953): Investigations into Yellow-leaf Disease of Phormium. III. Anatomical Aspects. *N. Z. J. Sci. Tech. A34* (*Supp. 1*): 17-30.

Roach, W. A. (1949): Plant Injection as a Physiological Method. *Ann. Bot.* (*n.s.*) *3*, 155-226.

*The writer is greatly indebted to Dr. F. J. Newhook for this examination and his detailed notes.

INVESTIGATIONS INTO YELLOW-LEAF DISEASE OF PHORMIUM

III. ANATOMICAL ASPECTS

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Summary

Phloem necrosis and xylem gummosis occur extensively in the rhizomes of yellow-leaf diseased phormium plants; these symptoms occur less regularly in leaves and are rare in roots. The vascular inclusions in diseased plants resemble wound gum in appearance and staining reactions, although they differ from it in that they occur more extensively and are not related to obvious local injuries. Observations on the time of appearance and distribution of the vascular symptoms, and experiments on the uptake of dyes, showed that these symptoms regularly preceded lateral and main root collapse, rhizome rot, and leaf symptoms.

INTRODUCTION

IN A preliminary note Boyce *et al.* (1951) reported that the cause of yellow-leaf disease of phormium was a virus transmitted by a plant hopper (*Oliarus atkinsoni*), by mechanical means, by root grafting and probably also by seed. As reported there, Strzemienski showed that translocation of dyes and radioactive phosphorus (P^{32}) from roots to leaves was prevented by blockage, apparently in the rhizome of diseased plants; Newhook showed that this blockage was caused by xylem gummosis usually associated with phloem necrosis, and that these symptoms preceded root and rhizome rot, leaf-yellowing, wilting and death of the plant. The history of yellow-leaf investigations and a description of the symptoms have been given in the first paper of this series (Boyce and Newhook, 1953). The second paper (Strzemienski, 1953) describes the investigations which initially demonstrated the translocation blockage. The present paper deals with the anatomical changes in the vascular system and their relationships to the other symptoms of yellow-leaf.

Atkinson (1921) and Cheeseman (1925) have described the general morphology of phormium, and Arber (1925) has given an account of seedling and leaf morphology. A phormium plant is composed of several shoots known as "fans", each of which has a life of from four to five years. Fans consist of a stout rhizome with leaves arranged in a tight distichous phyllotaxy with leaf bases which completely ensheath the rhizome. Leaves are semi-ensiform, with an open sheath at the base, a central concrescent region and a flat limb. They commonly reach a length of 8-12 ft in *Phormium tenax*, though in *P.colensoi* seldom half that length. Rhizomes may attain a length of from 6 in. to well over 1 ft before producing a tall terminal inflorescence; as this ripens, all leaves on the rhizome die away. During its life a fan produces several subsidiary fans from lateral buds, usually borne asymmetrically in the sheathing leaf bases in such a way that growth of the plant proceeds away from the centre. The roots, which may function for one or several years, bear numerous fine laterals. They may originate anywhere on the surface of the rhizome from root initials formed at the point of differentiation of the rhizome. These initials may remain dormant for several seasons or develop relatively early, forcing their way through the enveloping leaf bases.

Materials and Methods

Most of the diseased material was obtained at Moutoa from plantations of *P.tenax*, mainly of the cultivars '56' and 'Ngaro'. Other material used included cultivars '301', '273', '88' and various *P.tenax* x *P.colensoi* hybrid lines. Healthy material of '56S$_1$', 'Ngaro' and '88' was obtained from mature plants growing at Plant Diseases Division, Auckland.

Following the principles employed by Waters and Atkinson (1922) and Strzemiénski (1952), observations on vascular distribution and xylem translocation were made using 2 per cent naphthalene green G.S. (I.C.I.) and acid fuchsin (B.D.H. Standard Stain). Test tubes of the dye were applied to the cut ends of truncated roots for periods of 24-72 hours or applied as described later to transversely cut surfaces of rhizomes. Roots and rhizomes were usually cut under water. Dye uptake was tested *in situ* in the field, a trench one to two feet from the base of the plant being dug to expose the roots and to allow the placing of the tubes. At the end of the application period, the plants were lifted and washed free from soil. Each fan was then split in a median longitudinal plane through the treated root base and the distribution of dye recorded.

Anatomical studies were made from hand sections of fresh material which were frequently compared with stained microtome sections of tissues fixed in formalin acetic-acid alcohol and embedded in paraffin wax. Sections were stained with safranin and light green in clove oil, with aniline blue or with phloroglucinol and sulphuric acid following the recommendations of Hutton (1948).

Anatomy

Since the anatomical accounts of Atkinson (1921) and Arber (1925) are confined to the leaf and seedling, the present investigation includes such details of the anatomy of the whole plant as are necessary for an understanding of the pathological changes occurring in infected plants.

Vascular distribution

The leaf traces follow the fundamental pattern described by Arber (1925) for monocotyledonous stems with massive conical apices. After the traces enter the rhizome from the leaf base, the larger ones continue well in towards or across the centre, before turning outwards and downwards obliquely and following a sinuate course with a certain degree of anastomosis towards the periphery of the vascular region of the rhizome. The smaller traces follow a similar course but penetrate less deeply and reach the periphery at a higher level than the larger traces from the same leaf. At the peripheral region the larger, essentially composite leaf traces split into their component units. These, with the simple smaller traces, form a zone, 5-10 mm wide, of tightly packed small bundles immediately under the cortex. Anastomoses also occur in this zone, the lignified parenchyma of the outermost bundles of which are fused into a thin continuous layer. If any arc be described through the peripheral zone then the bundles on this arc will include trace units from the larger (adaxial) leaf bundles of younger leaves, and traces from smaller leaf bundles of older leaves.

Arber does not discuss for this type of monocotyledon the origin of roots on the rhizomes, nor the complex connections between root and leaf traces. In phormium, root initials and their vascular connections are apparently all laid down by the apical meristem of the rhizome. Where

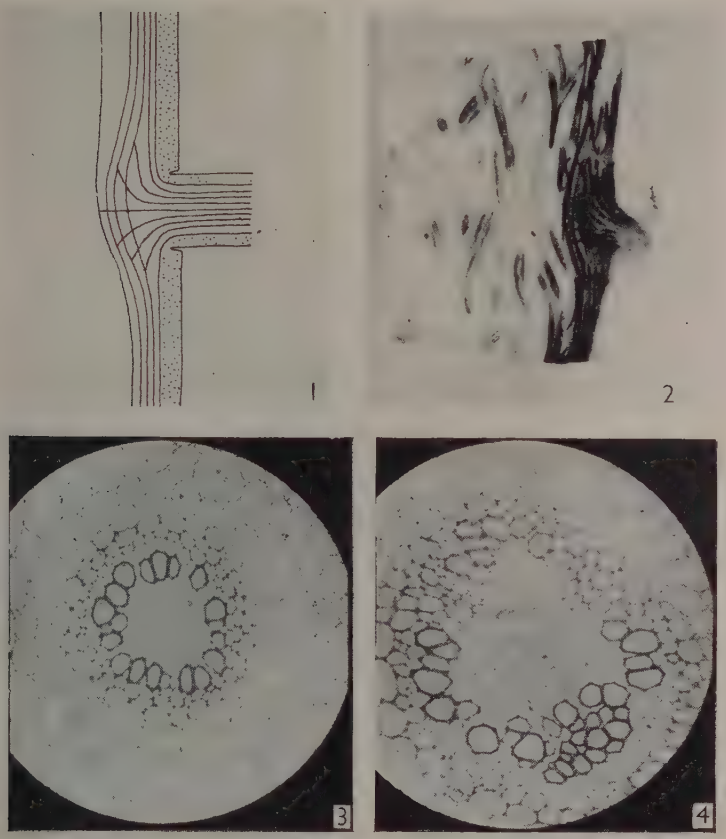


FIG. 1. Diagrammatic representation of root trace connections with peripheral rhizome bundles, seen in radial longitudinal section.

FIG. 2. Radial longitudinal section through portion of a rhizome and a root base; rhizome treated with 2 per cent napthalene green x 3½.

FIG. 3. Transverse section amphivasal bundle from healthy rhizome (safranin and light green, 3 x orange filter) x 180.

FIG. 4. Transverse section composite bundle with two units, healthy rhizome (safranin and light green, 3 x orange filter) x 170.

the root leaves the rhizome, vascular strands from all layers of the peripheral zone converge from every direction to form the root stele; xylem elements of the more central regions of the stele are connected with root traces arising as branches from deep-seated peripheral zone bundles. The more superficial elements on lateral and lower aspects of the root stele are connected with root traces branching from less deep-seated peripheral bundles, while elements of the upper part of the root stele continue upwards in the rhizome to constitute new peripheral traces in the outermost layer (Figs. 1 and 2). Any one root thus effects vascular connection with the traces of several leaves of a fan, and, if it lies below a lateral shoot junction, with the traces of leaves of more than one fan, since the shoot traces are ultimately connected to the peripheral bundle system in simi-

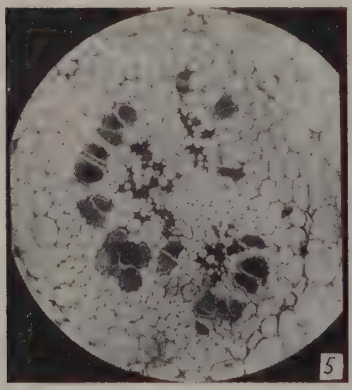
lar fashion to leaf traces. Each root trace usually connects to only one side of a rhizome bundle, hence, in translocation experiments, the commonly observed restriction of dye to a few of the vessels in the leaf bundles as reported by Strzemienski (1953). In many of the anastomoses in the rhizome, the fusing strands maintain their identity in the composite bundle until they separate again at a higher level.

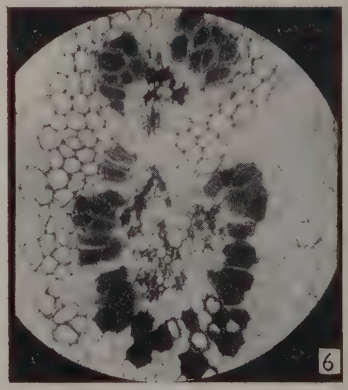
Histology

(1) *The rhizome*

(a) *Healthy.* The bulk of the rhizome internal to the leaf bases and the relatively narrow (3-5 mm) cortex is occupied by the vascular system embedded in storage parenchyma. Bundles in the peripheral zone defined above are crowded, amphivasal and of relatively small diameter; a single ring of vessels and tracheids surrounds a phloem consisting of sieve tubes, companion cells and thin-walled parenchyma. A few lignified parenchyma cells surround the bundle (Fig. 3). Most of the bundles internal to the peripheral zone are composite bundles (Fig. 4) with 2-4 or more crescent-shaped xylem groups.

(b) *Diseased.* Vascular bundles with extensive reddish gum deposition had been recognized early in the investigation as characteristic of the rhizomes of infected plants of some but not all varieties. Their frequency and intensity of development varied considerably from plant to plant. Isolations and direct examinations had failed to demonstrate the presence of micro-organisms in the reddened bundles. After Strzemienski (1953) had demonstrated vascular blockage in diseased plants, attention in this investigation was redirected to the rhizome bundles. It was found that phloem necrosis and xylem gummosis (Figs. 5 and 6) occur extensively in the rhizomes of yellow-leaf plants of all varieties, including those in which the red bundles are absent or rare. The red bundles constitute an extreme stage in vascular degeneration, a pale straw colour being more typical.





FIGS. 5 and 6. Transverse section composite bundles, diseased rhizome, showing phloem necrosis and xylem gummosis (safranin and light green, 3 x orange filter) x 180.

In xylem gummosis the vessels become completely filled with gum which may be coarse in texture or finely granular and almost homogeneous. In unstained sections the walls and contents are at first a pale straw colour, in some varieties becoming darker and turning eventually a dense orange-red. In its very earliest stages the gum stains with light green but not with safranin, though very soon a stage is reached when it takes the latter stain even more readily than do the vessel walls. The gum stains pink to bright red with phloroglucinol and acid.

Phloem necrosis at first involves only the sieve tubes and companion cells. These cells collapse and become filled with gum-like material. The colour and staining reactions are the same as for xylem gummosis.

In the final orange-red stage of gum deposition, all parenchyma cells within the affected bundles, and even those cells immediately surrounding them, may be involved (Fig. 7).

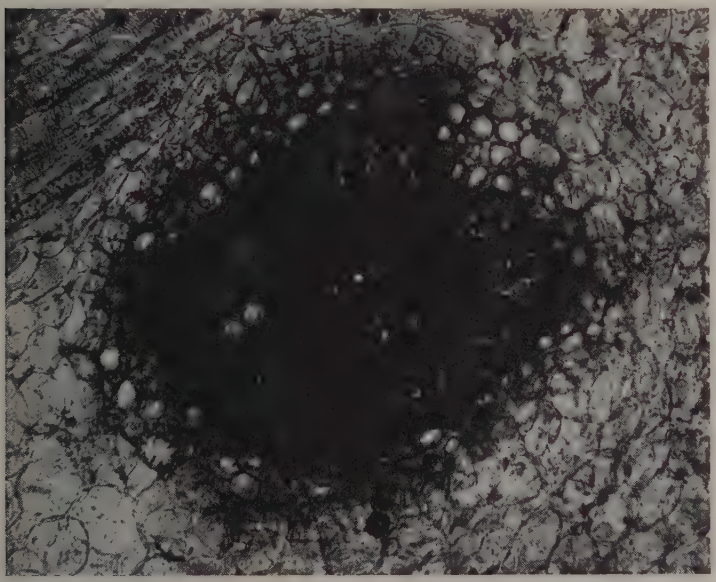


FIG. 7. Transverse section red vascular bundle, advanced stage of degeneration, gum deposition extending to all elements in the bundle and bundle sheath; (unstained) x 190.

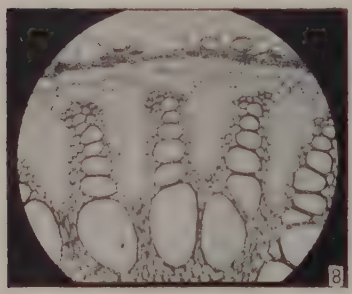
Phloem necrosis and xylem gummosis usually occur together, but sometimes a few strands may exhibit only the one or the other condition. The traces of older leaves may continue to be affected even after they enter the leaf bases, but, even in the very late stages of rhizome collapse, degeneration of the younger leaf traces in the rhizome seldom extends into the zone of cell expansion. In the lower parts of this zone in advanced cases, however, deposition of gum in some vessels and/or necrosis of some phloem cells may occur before lignification of the xylem has commenced.

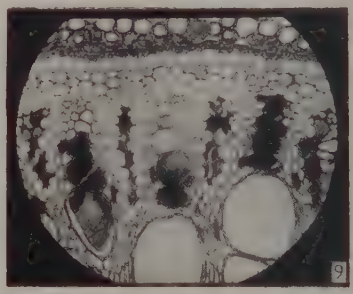
Vascular degeneration is not necessarily distributed uniformly throughout infected plants, e.g., in a single rhizome it may be confined to the zone immediately below the region of cell expansion or to one side: it may occur in some shoots but not others from a parent fan which itself may or may not show vascular degeneration.

In the advanced stage of yellow-leaf a general breakdown of rhizome tissues occurs, spreading upwards from the base or occasionally from one side. This is followed by invasion of saprophytic bacteria and fungi which complete the breakdown. The zone of rhizome rot may occasionally be sealed off temporarily by a darker layer of gum deposition involving all cells.

(2) *The root*

(a) *Healthy.* The stele has up to sixty or more alternating phloem and xylem groups embedded in heavily lignified fibres (Fig. 8). In the





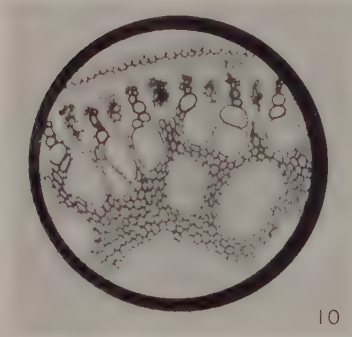


Fig. 8. Transverse section portion of healthy root stele (safranin and light green, 3 x orange filter) x 170.

Fig. 9. Transverse section portion of stele, root base, diseased fan, showing phloem necrosis and xylem gummosis (safranin and light green, 3 x orange filter) x 170.

Fig. 10. Transverse section portion of stele, distal portion of young root, diseased fan, showing phloem necrosis (safranin and light green, 3 x orange filter) x 80.

Fig. 11. Diagrammatic representation of three actual roots on diseased rhizomes, to show percentage of affected bundles in sections at positions marked with arrows.

main roots there is a central pith, though this is absent from lateral roots. There is a well-developed pericycle 1-4 cells wide which becomes sclerotic in older roots; an endodermis with heavy thickening on radial and inner tangential walls; an inner cortical zone with 1-4 layers of cells which frequently become heavily thickened; a wide zone of cortex commonly developed as aerenchyma; an outer cortical region of thick-walled cells, the outermost layer of which, the exodermis, has extremely thick outer tangential walls except in the passage cells; and a piliferous layer.

(b) *Diseased.* In the large number of roots examined, phloem necrosis and xylem gummosis did not extend from the affected rhizome bundles more than a few millimetres into the base of the root (Fig. 9), except in two cases where typical vascular degeneration was present throughout the length of fairly young roots (Fig. 10).

Sections were cut from large numbers of roots of different ages and at different distances from the rhizomes of plants in all stages of yellow-leaf. Fig. 11 illustrates the method of examining the roots. The three examples were all from Cultivar '56', and fans showed moderate to severe external symptoms. Arrows indicate where sections were examined after staining with phloroglucinol and acid. The figures refer to the percentage of rhizome bundles with degeneration, or of necrotic root phloem groups. In the case of the rhizome bundles the first figure refers to the deeper leaf traces, the second to the peripheral bundles. All lateral roots in these three examples were healthy.

With very few exceptions, abortion and death of roots was confined to those rhizomes or portions of rhizomes where vascular degeneration was present in a large proportion of bundles.

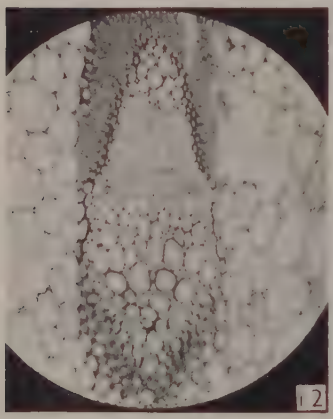
(c) *Occurrence of mycorrhiza.* In both diseased and healthy plants throughout New Zealand, endotrophic mycorrhiza of the vesicular-arbuscular or *Rhizophagus* type occur in the lateral roots and occasionally in main roots. Waters and Atkinson (1922) appear to have confused this endophyte with the *Ramularia* (*Cylindrocarpon*) sp. frequently isolated by them from healthy and diseased phormium roots and suspected as the cause of yellow-leaf.

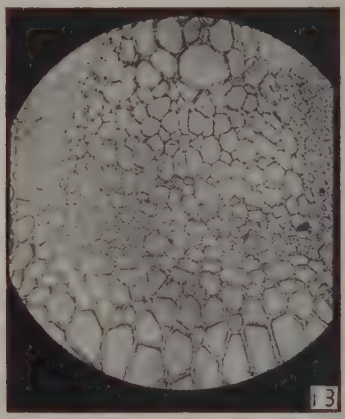
(3) *The leaf*

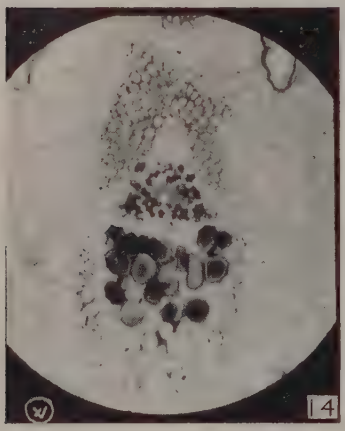
(a) *Healthy.* The general arrangement of the bundles in the limb and base is as shown by Arber (1925). Atkinson (1921) described the anatomy of the expanded portion or limb. The anatomy of the base is essentially different from that of the limb only in the arrangement of the bundles and fibre strands. The bundles (Fig. 12 and 13) are collateral throughout with xylem and phloem enclosed by heavy fibre sheaths (the fibres of commercial use). The protoxylem and protophloem tend to collapse, the latter becoming lignified in older and larger bundles. Fine lateral bundles connect the main strands.

(b) *Diseased.* In diseased fans, phloem necrosis and xylem gummosis frequently occur in older leaves but are not present in the youngest leaves. The staining reactions of affected tissues are the same as for affected rhizome bundles (Fig. 14). The symptoms occur less

regularly than in the rhizome and may be absent even from leaves in an advanced stage of yellowing. In the youngest and frequently the other leaves, bundles may show no degeneration throughout their length, whereas in their continuation as leaf traces in the rhizome they show severe phloem necrosis and xylem gummosis. When most bundles in the limb are affected, vascular degeneration may be infrequent or absent in the basal region. Although phloem necrosis (Fig. 15), or more rarely xylem gummosis, may occur alone in the leaf, phloem and xylem symptoms usually occur







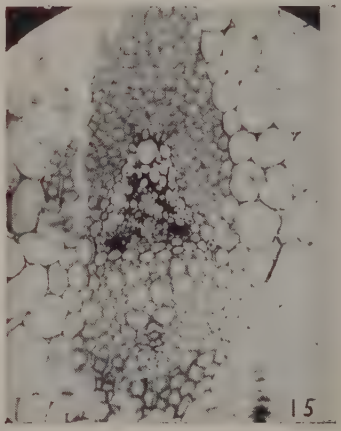


FIG. 12. Transverse section vascular bundle, healthy leaf (safranin and light green, 3 x orange filter) x 160.

FIG. 13. Transverse section phloem and portion of xylem, healthy leaf (safranin and light green, 3 x orange filter) x 300.

FIG. 14. Transverse section diseased leaf bundle (safranin and light green, 7 x green filter) showing affected phloem and xylem elements stained green (lighter) and red (darker) x 200.

FIG. 15. Transverse section diseased leaf bundle, necrotic phloem stained with light green (safranin and light green, 3 x orange filter) x 240.

together. Conduction of dye by yellowed leaves of diseased fans, an observation which gave rise to the earlier belief that no blockage existed in these leaves (Strzemienski, 1953), is explained by the fact that vascular degeneration may be absent from all or most bundles, phloem necrosis may occur alone, or xylem gummosis involve only a few of the vessels of affected bundles.

(4) *Causes of vascular degeneration other than yellow-leaf*

(a) *Miscellaneous.* There is no periderm formation in phormium; instead, wounds in all parts of the plant are protected by the deposition of gum in adjoining tissues. This deposition, especially in the leaves, frequently extends into the phloem and xylem a few millimetres further than in other tissues, and is indistinguishable in appearance and staining reactions from the gumming associated with yellow-leaf. The latter differs from the normal wound reactions only in its extent and its occurrence without relationship to obvious local injuries. Wounds on healthy rhizomes most commonly occur after natural death of roots, leaves and inflorescences; rhizomes frequently suffer gross physical injury during the subdivision of plants for vegetative propagation. On the roots of healthy plants cortical lesions with and without evidence of insect damage are of fairly frequent occurrence. Leaf lesions are caused by frost and by several species of fungi; physical injuries are brought about by cutting operations and by certain chewing insects. Where senescent leaves first turn yellow and then die, no vascular reaction has been observed in either yellow or dead regions; but there is a general pale brown staining of all cell walls and a tendency for phloem walls to stain faintly pink with phloroglucinol. Where death is more rapid and the transition is immediately from green to brown, gum deposition in phloem and xylem has occasionally been observed.

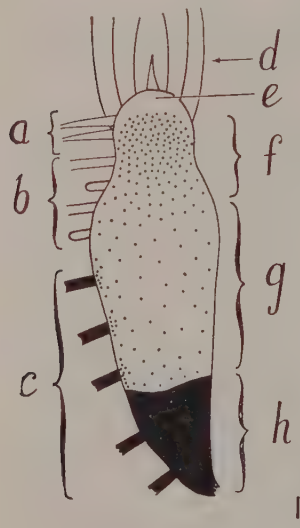
(b) *Desiccation.* Recent work (Boyce, 1953) has shown that summer soil moistures in parts of the Moutoa Estate are low. The effects of desiccation on the vascular tissues were therefore investigated. Examinations of excavated fans left exposed for long periods failed to disclose vascular symptoms comparable with those of yellow-leaf rhizomes. In one experiment fans from a single large plant were divided into three groups and were replanted either in soil or in infrequently watered sand, or were left exposed. The older leaves in all cases yellowed and wilted; the new leaf growth of plants in sand was foreshortened and apically blunted. These external symptoms are the same as those of yellow-leaf, but no corresponding rhizome symptoms developed during a period of nine months.

RELATIONSHIP OF VASCULAR DEGENERATON TO OTHER SYMPTOMS OF YELLOW-LEAF

The relationship of vascular degeneration to other symptoms was investigated by means of observations on its occurrence in diseased rhizomes in relation to organs showing other symptoms, and by means of tests with dyes.

(1) *Observations on the occurrence of vascular degeneration*

Fans of different varieties were examined. Fig. 16 illustrates in longitudinal section a typical infected fan. The region of maximum development of phloem necrosis and xylem gummosis lies behind the zone of differentiation and cell expansion. Vascular degeneration is sometimes confined to this region, but usually the bundles below it are almost all affected though pigmentation is less intense. In advanced cases of yellow-leaf, rhizome rot develops and advances upwards from at or near the base. The distribution of dead and dying roots is shown in Fig. 16. New roots are usually confined to the rhizome above or level with the top of the region of vascular degeneration. Unblocked bundles from the upper traces of the new roots skirt this region. Roots entering the rhizome immediately below the zone of maximum degeneration frequently have many dead laterals and lesions on the main root; others are represented by short abortive stumps. In both cases the root traces from all aspects of the root stele are almost completely blocked. Fan buds with traces entering the region of affected bundles show bud scale breakdown and rot, or in slightly older shoots, leaf yellowing and wilting. In the upper region the rhizome diameter is frequently reduced. There are fewer functional leaves, these being restricted to the uppermost region of the rhizome, the main traces of the outer, yellowing leaves passing rapidly to the centre of the region of maximum vascular degeneration.



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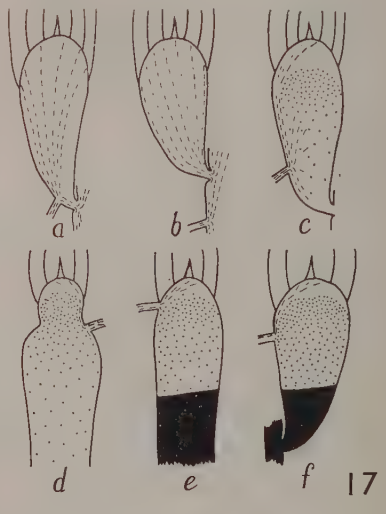

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Fig. 16. Diagrammatic representation of a median longitudinal section through a yellow-leaf infected fan: (a) tapered new roots, (b) abortive roots and roots with lateral rootlet death, (c) dead roots, (d) leaves, (e) rhizome apex, (f) region of maximum development of vascular degeneration, (g) region of rhizome with moderately affected bundles, (h) rhizome rot.

Fig. 17. Diagrammatic representation of median sections through bases of healthy (a, b) and diseased (c-f) fans. Dotted areas indicate distribution of vascular degeneration; broken lines show the distribution of dye-conducting bundles within the rhizome; rhizome rot is shown black.

TABLE I. CONDUCTION OF DYE FROM TRANSVERSELY CUT SURFACES OF PHORMIUM RHIZOMES

Fan No.	Yellow-leaf diagnosis prior to lifting	No. and condition of leaves	Rhizome rot	Red Vascular bundles	Bundles with suspected degeneration	Estimated percentage of vascular bundles with dye at different distances							
						Central				Peripheral			
						4mm	8mm	12mm	Apical region	4mm	8mm	12mm	Apical region
11	nil	8, relatively short, green	nil	-	—	100	100	50	50	100	100	50	50
8	nil	8, normal	nil	—	—	100	90	90	90	100	90	75	50
7	nil	13, normal	nil	—	—	100	90	50	50	90	90	50	50
10	nil	7, normal	nil	—	—	100	75	50	50	100	100	50	50
5	incipient	6, short, slight foreshortening and apical bluntening	nil	+	++	75	10	1	0	75	25	5	0
9	incipient?	7, mainly normal, 1 leaf with slight foreshortening and bluntening	nil	+	+	25	1	0	0	25	5	1	0
6	incipient?	6, slight apical bluntening, 2 yellow tips	nil	+	++	1	0	0	0	0	0	0	0
1	moderate	7, short, some yellowing	slight	+	++	10	1	0	0	50	10	1	0
3	moderate	5, green but short and blunt	moderate	+	+++	1	1	0	0	25	25	10	5
2	moderate	5, yellowed and dead tips	nil	++	++	1	0	0	0	10	1	0	0
12	moderate	5, yellowed and blunt	moderate	—	+++	1	0	0	0	10	1	0	0
4	severe	4, yellowed and blunt	severe	+	+++	10	1	0	0	25	1	0	0

"+, ++, +++"—relative amounts of vascular degeneration estimated before application of dye.
"—"—vascular symptoms absent.

(2) *Tests with dyes*

The following experiments with dyes were carried out to determine the degree of effective blockage present in fans at various stages of yellow-leaf with varying degrees of obvious or suspected vascular degeneration.

(a) *Gross rhizome translocation.* After preliminary tests to establish technique, twelve diseased fans of Cultivar 273 were lifted and split in a median longitudinal plane perpendicular to that of the fan. Leaves were left intact. The half-rhizomes were cut transversely through a band of whiter tissue which regularly occurs 4-7 cm below the apex of the rhizome, and which coincides approximately with the centre of the region of maximum vascular degeneration. A record was made of the relative numbers of obviously discoloured bundles and those merely suspected of vascular degeneration. Both surfaces of the transverse cut were repeatedly flooded with a 2 per cent aqueous solution of napthalene green for three minutes and were then left for 1½-2 hr, when the rhizomes were cut transversely at 4, 8 and 12 mm. from the original cut surface, and also immediately behind the apex. The percentage of vascular bundles carrying dye was estimated and recorded. Downward movement of dye gave figures which corresponded to those for upward movement, but only the latter are presented in Table I.

As seen from Table I the ability of the bundles to conduct dye was closely correlated with the amount of degeneration. The presence of xylem gumming and phloem necrosis in bundles suspected of degeneration was confirmed by examination of phloroglucinol-stained sections. Internal symptoms and reduced conduction were observed in two cases (6 and 9) diagnosed as "incipient?" before lifting for this experiment.

Similar results were obtained from tests on fans of other varieties with severe, moderate and incipient top symptoms.

(b) *Translocation from single roots.* The earliest tests with dyes and P^{32} (Strzemienski, 1952) had been carried out in the late summer of 1950, a time when diseased plants show maximum expression of the secondary, external symptoms. Dye and P^{32} movement from roots to leaves at this time was almost negligible in amount. During the autumn and winter, a partial and temporary recovery occurs on diseased fans; there is usually considerable production of new roots although these roots are weaker and shorter than those of healthy fans. *In situ* dye tests with naphthalene green and acid fuchsin were made in early spring of 1950 on several healthy and diseased fans, using roots which, from their appearance, had been produced in the preceding autumn. Although some of these were already showing signs of lateral rootlet death, dye conduction by these roots on diseased fans was often considerable. Fig. 17 illustrates examples of representative reactions.

Fig. 17 shows: (a) A typical healthy fan with treated root relatively low on the rhizome. Dye moved downwards to some extent, even into the parent rhizome, and upwards in an expanding cone to enter several leaf bases.

(b) A healthy mature fan on a healthy parent rhizome, with the treated root low down on the latter. Dye was present in some vessels of almost all bundles and all leaf bases.

(c) A fan in an early stage of infection, with most roots normal and no rhizome rot. Dye movement was partially restricted in the lower part of the rhizome and completely restricted in the region of maximum vascular degeneration.

(d) A diseased fan with very early leaf symptoms but many roots dead and dying. Dye movement was confined to the new traces immediately under the cortex, skirting the region of vascular degeneration. Downward movement of dye ceased at the junction of the lower root traces with affected bundles of the rhizome periphery below the root-entry.

(e) A severely diseased fan with only four functional, somewhat yellowed, short, blunt leaves remaining. Most roots were dead, and laterals on the treated root were beginning to collapse. Rhizome rot was well advanced. Dye movement was similar to (d).

(f) A severely diseased fan with five yellowed and two green leaves and basal rhizome rot. A few roots at the upper level were healthy but short and tapered; these opposite the zone of vascular degeneration had many dead laterals; roots 6 cm or more below the rhizome apex were dead. Dye uptake by the treated root was confined to the more superficial vessels of a strip comprising less than half the circumference of the stele. Lateral roots on this strip were normal, those on the portion not conducting dye being dead although the main root itself appeared to be quite normal. The dye-conducting portion of the stele was uppermost where it entered the rhizome and dye was present in the traces from it skirting the affected bundle zone and in the bundles of several leaves.

Several similar instances were noted of this restriction of lateral rootlet death to a non-conducting portion of a root associated in the rhizome with a zone of vascular degeneration.

(c) *Seasonal changes.* Similar observations to the above were made on many fans of different varieties during the spring and summer while external symptoms were increasing in severity. In one experiment *in situ* dye tests were made on twelve selected yellow-leaf plants in a Cultivar '56' plantation at Moutoa. The plants were divided into three similar groups which were subjected respectively to *in situ* dye tests in September, December and February. Dye was applied to 4-5 roots of all ages on each plant, except in the case of the final group when there were only 14 treatable roots available amongst the four plants.

In each plant at the commencement of the experiment, 3-5 fans were severely affected by yellow-leaf while an equivalent number showed very slight external symptoms. By mid-December, the eight remaining plants were showing slight or moderate yellowing, while by late February all except two fans in the last group of four plants showed very severe yellowing and wilting. Detailed results showed that sap flow, as indicated by dye translocation from roots, was increasingly restricted between spring and autumn; there was a strong correlation between external symptoms, restriction of sap flow and the development of internal symptoms. Restriction of the pattern of dye distribution in the rhizome was closely correlated with the presence of demonstrable xylem gummosis.

Similar results were obtained from other tests during the same period.

Discussion

Bawden (1950) and Esau (1948) have reviewed the literature relating to the nature of phloem necrosis and xylem gummosis. Phormium yellow-leaf resembles a number of virus diseases in which vascular degeneration is considered to be a primary symptom preceding such symptoms as death of lateral and main roots, loss of vigour, wilting and death. Such diseases include: American elm disease and quick decline of citrus with phloem necrosis: Pierce's disease of the grapevine, lucerne dwarf and phony peach disease with xylem gummosis: and black-root disease of snap beans with both phloem and xylem symptoms.

Schneider (1945) and Esau (1948) discussed the apparent identity with wound gum of the pathological phloem and xylem inclusions found in certain virus diseases. In phormium, the phloem necrosis and xylem gummosis are similar in appearance and staining reactions to, but are more extensively distributed than, localised responses to injuries. They are, however, less extensively distributed than the virus itself as determined by mechanical transmissions (Boyce *et al.* 1953).

Direct observations and the dye translocation tests showed that vascular degeneration in the rhizome precedes all other detectable symptoms of yellow-leaf, including bundle degeneration in the leaves. Whatever earlier activity of the virus leads to the appearance of phloem necrosis and xylem gummosis, these two phenomena, from their extent, distribution and relative time of occurrence, would appear to be sufficient in themselves to account for the collapse of the root systems and for the other secondary symptoms. The only vector so far discovered, *Oliarus atkinsoni* Myers, (Boyce *et al.*, 1951) is a phloem-feeder. Although this fact might suggest the phloem as the first site of virus activity, no evidence was obtained in this investigation that either vascular symptom appeared regularly before the other.

Acknowledgements

The writer wishes to record his appreciation of the co-operation of the various workers associated with the yellow-leaf problem, in particular Mr. W. R. Boyce and Mr. J. A. Swindlehurst, Botany Division, Department of Scientific and Industrial Research. Thanks are due to Mr. L. H. Millener, Auckland University College Botany Department for criticism of portions of the manuscript.

References

Arber, A. 1925: Monocotyledons. C.U.P., Cambridge. 258 pp.
Atkinson, E. H. 1921: *N. Z. J. Agric. 22*: 203-16.
Bawden, F. C. 1950: Plant Viruses and Virus Diseases, 2 ed. pp. 285-7. Chronica Botanica.
Boyce, S. W., Boyce, W. R., Chamberlain, E. E., Cumber, R. A., Fry, P. R., Matthews, R. E. F., Newhook, F. J. and Strzemienski, K. 1951: *N. Z. J. Sci. Tech., A33(3)*: 76-7.
Boyce, W. R., Chamberlain, E. E., Fry, P. R., Matthews, R. E. F. and Newhook, F. J. 1953: *N. Z. J. Sci. Tech. A34* (*Supp. 1*): 41-46.
Boyce, W. R. and Newhook, F. J. 1953: *N.Z. J. Sci. Tech. A34*(*Supp. 1*): 1-11.
Cheeseman, T. F. 1925: Manual of the New Zealand Flora, 2nd ed. pp. 320-1. N. Z. Govt. Printer, Wellington.
Esau, K. 1948: *Bot. Rev., 14*: 413-449.
Hutton, E. M. 1948: *Austr. J. Sci. Res., B1*: 416-38.
Schneider, H. 1945: *Phytopath., 35*: 610-35.
Strzemienski, K. 1952: *N. Z. J. Sci. Tech. A34* (*Supp. 1*): 12-16.
Waters, R. and Atkinson, E. H. 1922: *N. Z. J. Agric. 24*: 27-32.

INVESTIGATIONS INTO YELLOW-LEAF DISEASE OF PHORMIUM

IV. EXPERIMENTAL INDUCTION OF YELLOW-LEAF CONDITION IN *Phormium tenax* Forst. by the INSECT VECTOR *Oliarus atkinsoni* Myers. (HEM., CIXIIDAE.)

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Summary

(1) The yellow-leaf condition of *Phormium* has been recognized for many years, but only recently has transmission of the disease been obtained.
(2) The original experimental work, showing that the condition may be induced by insect feeding, is described.
(3) Subsequent experiments indicating the probability that the condition produced by the bug *Oliarus atkinsoni* is due to the transmission of a virus rather than to the injection of toxic substances, are detailed.

INTRODUCTION

IN JUNE 1949, the author commenced an intensive survey of the insect species associated with *Phormium* areas. This survey, which continued for more ttan a year, was carried out to ascertain if the yellow-leaf condition of *Phormium* might be due to mechanical injury by insects, and also to determine possible vector species which might cause the condition by indirect means or through toxic effects. The survey gave no grounds for suspecting direct injury as the main causal factor. Transmission work, using likely vector species, commenced in November, 1949.

This account describes the original experimental work which showed that adults of the bug *Oliarus atkinsoni* could transmit the yellow-leaf condition, and subsequent experimental work which verified the original results and provided further information. A preliminary note indicating these results has already appeared (Boyce *et al.*, 1951).

The bug which was first noted at Moutoa early in November, 1949, was the first to be tested because it is mainly restricted to *Phormium* as a host plant, and was abundant in badly affected areas.

OUTLINE OF LIFE-CYCLE AND ECOLOGY OF *Oliarus atkinsoni* AT MOUTOA

Oliarus atkinsoni appears as an adult feeding upon the leaves of *Phormium* from November to March (Fig. 1). It is a relatively large insect 7-9 mm. long. In some areas the adults are very plentiful, and it is not uncommon to see as many as 20 individuals upon a single blade of the plant. Mating has been observed on the leaves, but females return to the base of the plant to oviposit. From 50 to 90 eggs are laid in small circular pads of the mealy substance produced by the females. These groups of eggs are found within the old dead leaf sheaths at the base of the plant. Several weeks after the eggs are deposited, the nymphs hatch out, and feed upon the roots of the *Phormium* which pass through the old dead sheaths. Nymphs produce large amounts of mealy substance—a filamentous fluff which lines their galleries. This substance is produced copiously by all nymphal stages, issuing dorsally

Fig. 1.—Adult of the Plant Hopper *Oliarus atkinsoni* Myers.

from three posterior segments as tuft-like tails of fine mealy strands. Periodically, this material is brushed off by the walls of the galleries (adjacent dead leaf bases, etc.) which become coated with it. The substance possesses water-rejecting properties and gives the galleries and nymphs themselves some measure of protection against water. The life cycle from egg to adult appears to occupy a period of two years. *Phormium* plants affected with yellow-leaf produce much basal dead leaf due to the increased rate of die-back, which may well favour the build-up of bug populations.

In the Manawatu district, it has been recognized for many years that the overdraining of land and the stopbanking of the river have resulted in the dwindling and receding of *Phormium* from those areas which no longer receive the periodical inundations. Under natural conditions nymphal populations of the bug are restricted to the better drained marginal bushes. At Moutoa, and in other areas, much of the *Phormium* no longer receives such inundations, and this has produced favourable breeding conditions for *Oliarus atkinsoni* over extensive areas.

Experimental Procedure and Results

Early in November, 1949, when adults of the bug first appeared, techniques for infection feeding of seedlings were tested and the first transmission trials commenced on 2 December.

Experiments 1 and 2 belong to a series set up in 1949 in which possible vector species were subjected to preliminary screening tests. Experiments 3 and 4 were set up in the following season to obtain additional information. In each of the experiments described here, the seedlings used were raised together from seed of the one variety of *Phormium.* In Experiments 1-3, glass-house raised seedlings were used in order to reduce the chances of inadvertent *Oliarus* infection. In Experiment 4 seedlings used were raised under field nursery conditions.

Experiment 1

Technique: In this experiment set up 2 December, 1949, 7- to 8-months-old seedlings, which had recently been transferred to pots, were used. There were 20 test plants and 20 control plants. The adult bugs were collected with an aspirator from *Phormium* bushes in which the majority of the fans showed yellow-leaf in a nearby plantation and were confined to the test seedlings by means of glass tubes of $1\frac{1}{2}$ in. diameter pressed into the soil around the base of the plant and plugged above with cotton-wool. The bugs thus had access to the leaf bases, upper roots (in some cases), and all but the distal portions of the leaves.

Ten bugs were placed on each of the 20 test plants, those bugs remaining alive at the end of 7 days being removed. The plants were then rested for one week when a further dosage of 7 bugs was given, those which remained at the end of 7 days being removed. Each test plant thus received a dosage of 17 bugs with a possible maximum feeding dosage of 117 bug-days. No replacement of bugs dying during test feeding was made. This experiment was set up in a glass-house, but later was removed to a shade-house, insect-proof covers being supplied to all plants while adults of the bug under test were still present in the field.

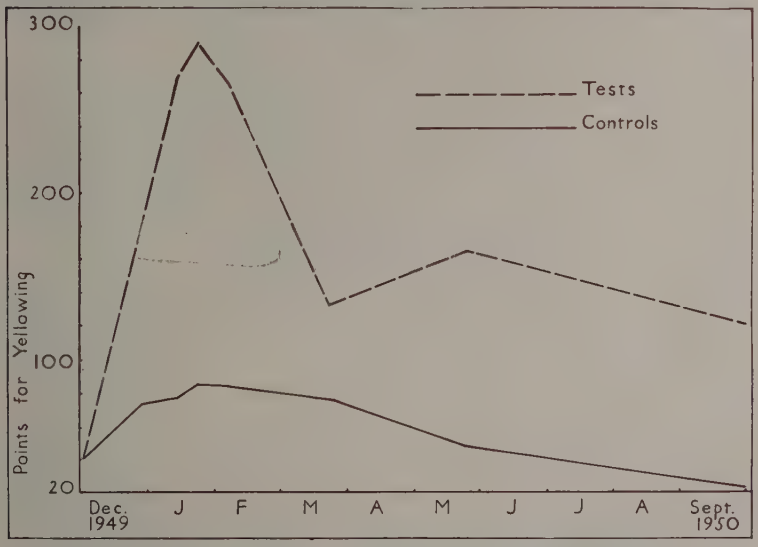

FIG. 2.—Relative Incidence of Yellowing in the 20 Test Plants and 20 Control Plants during the first 10 months of Experiment 1.

Results: In this experiment a marked difference between test and control plants was apparent by the end of the fourth week, the former showing considerably more yellowing. As the leaves usually yellow progressively from the tip, a measure of yellowing was determined for each plant by giving to each leaf (in order of age) an index of yellowing; an entirely green leaf was given 0 points, an entirely yellow leaf, 10 points, with estimated intermediate values for the other leaves. Fig. 2 indicates the relative incidence of yellowing in the test and control plants

during the first ten months of the experiment. The recording of changes was discontinued at this stage owing to the complication of assessment caused by stooling of plants and the death of the original fan in some instances. A record was kept of when new fans were produced. The removal of dead leaves was carried out and recorded so that it was known when all leaves present at the time of infection feeds had died.

Fig. 2 shows the marked increase in yellowing which appeared in the test plants, this reaching its maximum late in January, 1950. The control plants also showed some increase in yellowing, but to a much lesser degree; this was due no doubt to the fairly recent potting up. At this stage no symptoms other than that of excessive yellowing were used for diagnosis, and it was fully realized that several factors, e.g., feeding effects, toxins, etc., might be responsible for the disparity in test and control plants. From February to September, the period of new growth, there was a marked fall-off in yellowing of the test plants but only a gradual fall-off in the controls. By spring of 1950, however, the difference between test and control plants had become marked once more. The death of the older leaves in the test plants and the shortness of new growth had caused them to appear stunted. As the season proceeded, these differences became even more apparent and the excessive yellowing and die-back of test plants reappeared: at this stage it became apparent that a lasting systemic trouble had been induced. Leaves and fans produced several months after the bugs had been removed were showing the typical yellowing.

Meanwhile the work of Strzemienski (unpublished report, 13.4.50) had shown that . . . "there is some serious impairment of the ascent of solutes from roots to leaves in Yellow Leaf plants in the sense that in spite of roots of normal appearance and similar leaves there may be very little of root-to-leaf translocating ability left. This impairment may be due to some blocking of the translocation channels within the rhizome," and Newhook, using the phloroglucinol and acid technique, showed that it was possible to demonstrate a blocking of the phloem and xylem in the rhizome of yellow-leaf plants.

In making the final analysis of this and other experiments described here, full use has been made of the symptom of phloem and xylem blockage as shown by the phloroglucinol and acid technique, the rhizomes of all plants in the experiments (except where indicated otherwise) being sectioned and stained and microscopic examinations carried out.

In Experiment 1, 18 of the test plants remained alive at the time of the final examination (24.5.51), two having died at a considerably earlier date from causes undetermined. Sixteen of these plants showed typical external symptoms of yellow-leaf, together with phloem and xylem gummosis of the rhizome, the other two being unaffected. Nineteen of the 20 control plants were alive, and none showed external or internal symptoms of the yellow-leaf condition. One of the control plants had died at an early stage in the experiment from undetermined causes. Fans produced by test plants several months after the test feeding showed the external as well as the internal symptoms. Fig. 3, Plate 1, shows three infected test plants (L) and three control plants (R) from Experiment 1.

Experiment 2

Technique: In this experiment set up on 15.12.49, seedlings 7-8 months old and recently transferred to pots were grown in a shade-house, where

FIG. 3.—Comparative Growth of Infected, Non-infected and Control Plants. (The three left-hand plants in each plate (except Plate 2. are infected; all photographs were taken on 15/5/51.)

Plate 1. Infected test plants and control plants from Experiment 1, initiated 2/12/49.

Plate 2. Infected test plants and control plants from Experiment 2, initiated 15/12/49. (Note: the right-hand test plant is not infected.)

Plate 3. Infected and non-infected test plants from the 9 bug-day dosage trial of Experiment 4; initiated 13/12/50.

Plate 4. Infected and non-infected test plants from the 27 bug-day dosage trial of Experiment 4; initiated 13/12/50.

Plate 5. Infected test plants from the 54 bug-day dosage trial and control plants, from Experiment 4; initiated 13/12/50.

temperatures remained lower than those occasionally reached in the glass-house. It was considered that the occasional high temperatures of the glass-house might affect transmissions. Bugs were caged on test plants by means of fine wire-gauze tubes pushed into the soil around the base of the plants and plugged above with cotton-wool. Sleeves of cloth were suspended from above and tied around the pots to catch any bugs which might escape from the tubes. Three bug applications

were made to each of the 15 test plants. Each of these consisted of 8 bugs for a week, with a rest period of a week between dosages. All bugs remaining alive at the end of the week were removed. Each test plant thus received 24 different individual bugs and the possible maximum feeding dosage comprised 168 bug-days. No replacement of bugs dying during the test feeding was made. When the test feedings were completed, the gauze tubes were removed, but all plants were protected by the cloth covers which remained in position until the flight season of the bug had ended. The controls consisted of 15 plants protected in a like manner.

Results: The changes in the plants during the first 9 months of the trial followed closely those described in Experiment 1; the yellowing of test plants showed a gradual fall following the initial peak, there being no second minor peak as in Experiment 1 (Fig. 1, March). The control plants showed no increase in yellowing in the early stages such as occurred in Experiment 1. By February, 1951, the difference between test and control plants had become very marked, and it was apparent that many test plants were dying out. A final analysis of all plants was made on 31.5.51. Three test plants remained alive; two of these showed typical external and internal symptoms, while the third, although scarcely robust, had no yellow-leaf symptoms. This latter plant appeared to receive a set-back following the test feeds which at the time was thought to be due to fungal attack. The plant subsequently grew slowly but with no signs of yellowing, and when finally examined showed no external or internal symptoms. Thirteen of the control plants showed no external or internal symptoms. One died at an early stage in the experiment from undetermined causes; in another there was yellowing, but the rhizome was in too advanced a stage of decomposition to check for internal symptoms.

Fig. 3, Plate 2 shows the three remaining test plants (L) and three control plants (R) from Experiment 2. The right hand test plant is the unaffected one referred to above.

Experiment 3

Technique: This experiment was set up on 5.12.50 to ascertain if the yellow-leaf condition could be produced by using a much reduced number of bugs. Seven-months-old seedlings of a variety known as SS were used, and the technique followed that described in Experiment 1.

Results: The results (see Table I) of this experiment, finally analysed on 31.5.51, indicated that as few as two bugs are capable of producing full external symptoms and the internal necroses of the yellow-leaf condition in seedling material.

Table I. Details of Experiment 3

Bug Dosage	Number of Plants	Number of Bugs per Plant	*Number of Infected Plants	Plants dying in Expt. (Causes Undetermined)
1 Bug, 7 days 1 Repeat	15	2	4	1
8 Bugs, 7 days 1 Repeat	4	16	4	—
No Bugs Controls	14	—	1**	3

*Based on external and internal symptoms.
**This plant showed typical yellow-leaf symptoms.

TABLE II. DETAILS OF EXPERIMENT 4

Bugs Maintained on Plants	Total Bug-days	Number of Bugs Used per Plant	Number of Plants	Positive Infections Determination on		Percentage of Infected plants Determination on	
				A External Symptoms	B Internal Symptoms	A External Symptoms	B Internal Symptoms
1 Bug, 9 Days Once	9	1-3	52	14	14	27	27
3 Bugs, 9 Days Once	27	3-9	30	18	18	60	60
3 Bugs, 9 Days, Twice	54	6-12	30	19	20	63	67
8 Bugs, 9 Days, Twice	144	16-30	30	27	28	90	93
No Bugs Controls	—	—	56	0	0	0	0

NOTE: All plants were alive at date of final analysis, 5.5.51, although many test plants were severely affected.

Experiment 4

Technique: This experiment was set up on 13.12.50, in a glasshouse and later removed to an insectory. Eighteen-months-old well established seedlings of *Phormium* variety '301', which is known to be very susceptible to yellow-leaf disease, were used in pots. Bugs were enclosed on individual plants by means of wire-gauze cages which covered the whole plant and entered the soil around its base. During infection feeds, the number of bugs per plant was maintained at a known level, bugs which died or escaped being replaced. It was found that the female bugs which are larger and tend to live longer than the males were easier to maintain in the cages, and these were used during the latter half of the experiment. The exact number of bugs used per plant in each experimental group varies owing to different death rates and replacement rates for each plant, but the limits of the numbers used per plant in each group are indicated.

Results: The form of this experiment and the results obtained are indicated in Table II. The expression of external symptoms in infected test plants followed the usual course, although they became obvious several weeks earlier in the heavy bug-dosage groups. The number of infected plants has been obtained from: (1) the study of external symptoms of all plants, and (2) the internal symptoms indicated by the histological examination of a randomly selected half of the trials in each group and multiplying this figure by 2. It will be seen that the percentage of infected plants bears a positive correlation with the number of bugs used during test feeds.

In Fig. 3, Plate 3 shows three infected plants (L) and three uninfected plants (R) from the 9 bug-day dosage of Experiment 4; Plate 5 shows three infected plants (L) and three uninfected plants (R) from the 27 bug-day dosage of Experiment 4; Plate 5 shows three infected plants

FIG. 4.—The root systems of yellow-leaf infected and healthy plants.

from the 54-bug-day trial (L) and three control plants (R) from Experiment 4. These plates (3-5) indicate the complete expression of symptoms obtained at all bug-dosage levels. Fig. 4 shows the roots of yellow-leaf infected (L) and healthy (R) plants from Experiment 4. The majority of the roots in the infected plant have died back concurrently with the yellowing of the leaves.

An analysis of the total length of non-yellowed leaves per plant in Experiment 4 was made on 5.5.51, and this is summarized in Fig 5,

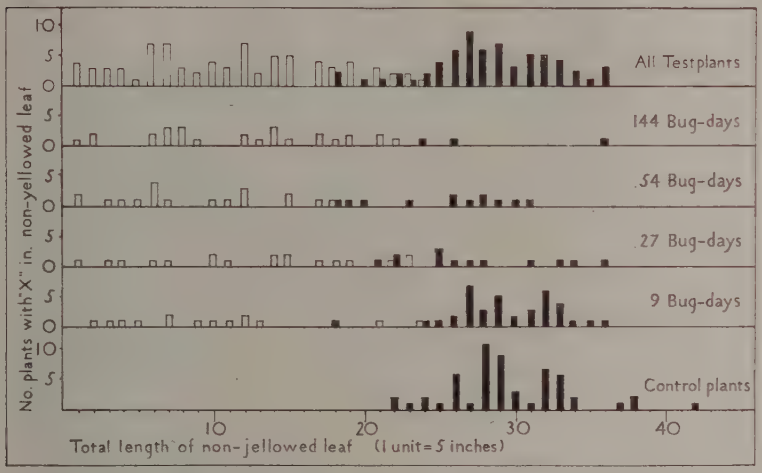

FIG. 5.—Frequency distribution of total length of entirely green leaves per plant at varying bug dosages and for all bug dosages combined in Experiment 4 (solid columns or frequency rectangles represent control plants and uninfected test plants, the open ones represent infected test plants).

which shows the frequency distribution of total length of entirely green leaves per plant, at varying bug dosages, and for all bug dosages combined. The distribution of diseased and non-diseased plants is shown. This is based on the diagnosis of yellow-leaf on external symptoms by two independent observers, the author and Mr. J. A. Swindlehurst who has had long experience of yellow-leaf in the field. A confirmatory histological examination of a randomly selected half of the trials in each group of Experiment 4 showed complete coincidence of diagnosis made by this method with those based on external symptoms.

The figure showing the total length of entirely green leaves per plant was obtained by summing the lengths of all individual leaves of the original fan (and only those leaves) which showed no yellowing. It is therefore a measure of the health of the plant. At the commencement of expression of symptoms of the yellowed condition, there appeared to be little difference in total length of leaf (green plus yellowed portions) of test and control plants. The measurement could possibly have been the complementary one, "total length of leaves showing yellowing", but owing to the rapid transition to the dry and shrivelled condition, it would have been difficult to distinguish between a dry leaf resulting from normal death and one resulting from the disease. It is probable that the normal variation in vigour, habit, and tendency to stool (only

the original fan has been analysed) have not allowed complete separation of infected and uninfected plants at the stage when analysis was carried out; nevertheless, there is distinct bimodality at each bug-dosage level and in the experiment as a whole.

Discussion

Experiments 1 and 2 set up in 1949 were successful in incriminating the vector *Oliarus atkinsoni*. Experiment 3 in 1950 showed that full symptoms could be produced by as few as two bugs. Experiment 4 in 1950 was designed to obtain information on possible toxic effects, but is not conclusive owing to the fact that, as indicated by Carter (1936), one may not assume that a toxin, when it exists, is a normal constituent of the saliva of all adult individuals of an insect species at the same time. Carter has shown the presence of toxic races of mealy bugs in his studies on pineapple wilt. In addition, the question of degree of resistance of individual plants from the one seed batch, if such exists, may have complicated results. This experimental work is necessarily of a preliminary nature, but it does incriminate the vector species, *Oliarus atkinsoni*, and suggests that the induced condition is probably due to the transmission of a virus; however, it does not rule out the possibility of transmission of other pathogens such as bacteria or fungi, or the possibility of the toxin hypothesis.

Studies on the biology and ecology of *Oliarus atkinsoni* and experimental work to determine virus-vector relationships continue. Additional possible vector species under test at present include another species of *Oliarus*, a species of *Erythroneura*, two species of mealy bugs, and a scale insect.

There appears to be only one other instance of the incrimination of a Cixiid. as a vector species. This is the case of a disease of tomatoes in Crimea. (Goldin and Parievskaia, 1950).

Acknowledgments

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References

Boyce, S. W., Boyce, W. R., Chamberlain, E. E., Cumber, R. A., Fry, P. R., Matthews, R. E. F., Newhook, F. J. and Strzemienski, K. (1951): Preliminary Note on the Yellow-leaf Disease of *Phormium*. *N. Z. J. Sci. Tech., A33(3)*: 76-77.

Carter, W. (1936): The Symbionts of *Pseudococcus brevipes* in Relation to a Phytotoxic Secretion of the Insect. *Phytopath, 26*: 176-83.

Goldin, M. I. and Parievskaia, A. P. (1950): Materials on Big Bug of Tomatoes in Crimea. Some Data on the Biology of Vector (*Hyalesthes obsoletus*). *Mikrobiologiia, 19*: 527-31.

INVESTIGATIONS INTO YELLOW-LEAF DISEASE OF PHORMIUM

V. TRANSMISSION EXPERIMENTS AND DISCUSSION ON CONTROL

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Summary

Experimental transmissions of yellow-leaf disease of phormium established that the causative virus is readily transmitted under suitable conditions by leaf rubbing with infected sap and by the hopper *Oliarus atkinsoni* Myers. Transmission through root grafts occurred in a few cases. There is some evidence that seed transmission occurs. The evidence suggests the virus is endemic to the New Zealand area.

It is likely that control measures will include: control of the insect vector by flooding and other means; elimination of sources of infection in nurseries and plantation areas; and breeding for resistance.

Introduction

Extensive mycological and physiological investigations on yellow-leaf disease of phormium have in the past given negative results. This paper describes transmission trials which established that the disease is caused by a virus.

Graft Transmission

Experiment 1: In September 1947, sixteen fans from a plant of *Phormium tenax*, probably cultivar '273'S_1, were root grafted to infected cultivar 'Ngaro' plants from Moutoa. Organic unions appeared to have been established in seven of the grafts and symptoms of yellow-leaf developed in three of these, after about thirty months, in the autumn of 1950. Sixteen ungrafted plants of the same cultivar growing in the same plot remain symptomless.

Experiment 2: In October and December 1949, root, lateral bud, and rhizome apex grafts were carried out between fans of healthy plants of cultivar '56'S_1 growing at Auckland, and yellow-leaf plants of cultivar '56' from Moutoa. In seven root grafts organic unions were established. In November 1951, after 23 months, one of these showed yellow-leaf symptoms.

Mechanical Transmission

In both experiments, inoculations were carried out by leaf rubbing with carborundum, leaves being washed in running water immediately after inoculation.

Experiment 1 (9/11/49): Test plants were eight-months-old seedlings of cultivar '301'. Infected plants of '273' were used as source of inoculum. From results in Table I it is evident that yellow-leaf is readily transmitted, at least in the early summer, in the expressed sap of leaf, root or rhizome of infected plants of this cultivar. Symptoms appeared either in the autumn of 1950 or were delayed until the following autumn or winter.
Experiment 2 (20/4/50): Test plants were thirteen-months-old seedlings of '301'. Inoculum was extracted from infected plants of '56' and 'Ngaro'. Results in Table I show that only one infection had occurred by November 1951 in the 72 plants inoculated.

TABLE I. TRANSMISSION OF YELLOW-LEAF TO P.TENAX BY LEAF RUBBING WITH CARBORUNDUM POWDER (Indicator: Cultivar '301'S_1.)

Date	Source of Inoculum	Plants Inoculated	Plants infected	Plants Infected after 4-6 months	New Infections after 16-21 months
9/11/49	Young leaves	6	6	2	4
	Old leaves	6	6	4	2
	Young and old leaves	6	6	2	4
	Rhizome apices	6	6	5	1
	Rhizomes with red vascular bundles	6	5	2	3
	Roots	6	5	1	4
	Abortive roots	6	6	0	6
	Necrotic lateral buds	6	6	0	6
	Root, rhizome and buds +0.5% Na_2SO_3	6	3	0	3
	TOTAL	54	49	16	33
	Per cent transmission	—	91	30	61
	Control-(water rubbed)	11	0	—	—
20/4/50	'56' Leaf	12	*0		
	Root	12	0		
	Rhizome	12	0		
	'Ngaro' Leaf	12	1		
	Root	12	0		
	Rhizome	12	0		
	Control-(water rubbed)	11	0		

*After 19 months

INSECT TRANSMISSION

As the result of a survey by Dr. R. A. Cumber, Entomological Research Station, of the insects associated with phormium areas, a number of likely species have been tested as possible vectors. So far the only insect found to transmit the disease is the plant hopper *Oliarus atkinsoni* Myers. Adults of this hopper appear, often in large numbers, from November to March, feeding on the leaves of phormium plants. Eggs are laid in the sheathing bases of old leaves and hatch after several weeks. The life cycle appears to be of two years' duration. Nymphs feed on roots at the base of plants. Cumber (1952) gives a full account of the life cycle and ecology of *O.atkinsoni*. Transmission experiments with *O.atkinsoni* have been carried out both at Moutoa and at Auckland where those described below were carried out.

Experiment 1 with *O.atkinsoni*: On 16/12/49, twelve adult *O.atkinsoni* collected from yellow-leaf plants at Moutoa were added to each of six pots containing three 8-months-old '301'S_1 seedlings. Plants were caged with muslin by the method described by Cottier (1931). After six days, when all hoppers were dead, cages were removed. Sixteen control plants, two or three per six-inch pot, were left uncaged.

Seven months after addition of insects, 15 of the 18 treated plants had developed symptoms of yellow-leaf. On 14/7/50, eight of these were lifted for observation. Internal symptoms were present in every case. The remaining 10 test plants and the 16 symptomless checks were transferred to a field plot on 5/10/50. Twenty months after exposure to insects, three test plants were dead, four showed severe

yellow-leaf symptoms and three still remained healthy. All 16 control plants were still symptomless.

Experiment 2 with *O.atkinsoni*: Six-months-old seedlings of '56'S_2 raised from seed collected from healthy plants at Auckland and growing singly in four-inch pots, were used as test plants.

Hoppers were collected both from yellow-leaf plants at Moutoa and from a stand of *P.tenax* at Lake Omapere, near Kaikohe, where no symptoms of yellow-leaf had been observed. Hoppers from Lake Omapere are referred to hereinafter as "healthy hoppers".

Insects were caged on plants with three-inch diameter plastic cylinders fitted with muslin tops. The experiment was set up in the glasshouse on 10/1/51. Survival of hoppers was noted. Cages were removed on the third day, by which time all hoppers had died. In early June all pots were removed to the field and plunged in the soil. Results, given in Table II, confirm the transmissions by *O.atkinsoni* obtained in the previous experiment and show that one hopper feeding for not more than two days is sufficient to transmit yellow-leaf. No yellow-leaf symptoms appeared in any of the plants on which healthy hoppers had been fed, or on caged or uncaged check plants.

TABLE II. TRIALS WITH ADULT *O.ATKINSONI* (Indicator: Cultivar '56'S_2.)

Source of hoppers	Hoppers per plant	Plants inoculated	Plants infected
Healthy *P.tenax*, Lake Omapere	1	20	0
	3	20	0
	8	20	0
Yellow-leaf *P.tenax*, Moutoa	1	20	2
	3	20	4
	8	20	5
Checks: No insects	Caged as for test plants	60	0
	Uncaged	25	0

Experiment 3 with *other insects*: Table III gives results of preliminary trials in which the aphid *Aulacorthum solani*, a collection of unidentified thrips, and the hopper *Sephena cinerea*, were tested for ability to transmit yellow-leaf from *P.tenax* to *P.tenax*. No transmission has been obtained in these experiments.

Root feeding cicada nymphs (*Melampsalta cruentata*) were also used in a series of transmission experiments at Moutoa from 1947 to 1949. Results from these trials were inconclusive owing to the appearance of the disease in untreated check plants as well as in the plants on which cicadas were fed. It is likely that the non-exclusion of leaf-feeding vectors from adjacent diseased plantation areas, accounted for these infections.

SEED TRANSMISSION

Three lines of seedlings were used in mechanical and insect transmission experiments at Auckland:

TABLE III. EXPERIMENTS WITH *AULACORTHUM SOLANI*, THRIPS AND SEPHENA CINEREA.

Date	8/12/49	23/6/50	10/1/51		
Insect	*A.solani*	Thrips	*Sephena cinerea*		
Source of insect	Healthy phormium seedlings	Yellow-leaf phormium	Yellow-leaf phormium	*Leptospermum scoparium*	
Source of infection	Yellow-leaf phormium 'Ngaro'	ditto	ditto	Yellow-leaf phormium	nil
Indicator variety	'301'S_1	'301'S_1	'56'S_2	'56S_2	'56'S_2
Prefasting time	18 hr.	nil	nil	nil	nil
Infection feeding time	10 min.	Indefinite	Indefinite	5 days	nil
Time on healthy plants	24 hr.	Indefinite	4-7 days	4-7 days	4-7 days
Number of Insects per plant	10	20	3	3	3
Number of Plants inoculated	6	18	5	16	20
Number of Plants infected	0	0	0	0	0
Number of Check plants	6	25	24		
Number of Check plants infected	0	0	0		

(1) '56'S_1. Seed was collected at Moutoa in February 1949 from apparently healthy plants of '56' which have since developed a high incidence of yellow-leaf. Seedlings were raised at Auckland.

(2) '56'S_2. Seed was collected in February 1951 from plants of '56'S_1 established at Auckland in 1946, which have shown no yellow-leaf symptoms. Seedlings were raised at Auckland.

(3) '301'S_1. Seed was collected at Moutoa in February 1949 from plants of '301', all of which have since developed yellow-leaf. Seedlings were raised at Moutoa but transferred to Auckland before the *O.atkinsoni* flight season.

In the first of these lines, a considerable number of infections occurred in the uninoculated checks as well as in a similar number of test plants. Experiments using this line of seedlings were therefore invalid and have not been detailed. The proportion of yellow-leaf plants occurring in uninoculated checks of the three seedling lines used at Auckland were:

'56'S_1	46/65
'56'S_2	0/189
'301'S_1	0/72

The only methods of infection for these plants would appear to be by some undetected insect vector, (*O.atkinsoni* has not been observed occurring naturally at Auckland) by mechanical contamination, or through the seed. The first two possibilities are made unlikely by the fact that while plants of all three lines have had similar treatment and were interplanted in the same block, infections in the checks have been confined to the one line, '56'S_1. Infections occurred consistently in both checks and treated plants in all experiments with this seedling line. It is, therefore, probable that yellow-leaf can be transmitted through the seed.

Transmission Trials With Indicator Hosts

To test the possibility that the virus causing yellow-leaf of *P.tenax* might be related to a known virus, the following attempts were made to transmit infection to a range of plant species recognised as indicators for a number of commonly occurring viruses. Twenty unidentified thrips occurring naturally on infected *P.tenax* at Moutoa were caged at Auckland on each of four White Burley tobacco, three tomato and two *Nicotiana glutinosa* plants. From six to twenty individuals of two species of aphid were transferred to test hosts after short feeding periods on yellow-leaf *P.tenax* 'Ngaro' plants; *Myzus persicae* to 50 cucumber and six tobacco; *Aulacorthum solani* to ten cucumbers, six French beans and eight broad beans. Sap from infected leaf, root, and rhizome of cultivars '56', '273' or 'Ngaro' was used to inoculate by leaf rubbing with carborundum powder, tobacco, *N. glutinosa*, *N. rustica*, tomato, cucumber, French bean, broad bean, swede and aster.

No evidence of disease transmission was obtained in any of the above trials.

Control Measures

Further information is needed before detailed control measures can be formulated. However, from the information already available it is clear that consideration should be given to control of the insect victor, elimination of sources of infection and breeding for resistance.

1. *Control of insect vector*
   (a) *By flooding.* Well substantiated observations have shown that rapid increase in the incidence of yellow-leaf frequently follows drainage of *P.tenax* areas. Winter flooding of a fifty acre trial area at Moutoa in two successive years 1949 and 1950, has shown that phormium withstands inundation to a depth of three feet, for periods up to four weeks during the winter months, and benefits from the resulting suppression of much weed and insect infestation. Observations by Cumber have shown that the nymphal stage of *Oliarus atkinsoni* is vulnerable to long periods of immersion. It is probable therefore that prolonged flooding, where possible, will be a desirable method of controlling the vector.
   (b) *By insecticides.* There are many situations in which flooding will be impracticable. Investigation will be necessary to ascertain whether application of insecticides will be of value in these cases.

2. *Removal of sources of infection*
   (a) The elimination of yellow-leaf will involve long term replacement of many existing *P.tenax* stands.
   (b) At the present time rapid spread of infection is taking place in the nurseries at Moutoa. An important aspect of control will be the raising of virus-free plants for planting. This should be possible by maintaining suitable isolation of nursery areas, by extensive roguing, and by the use of seed from virus-free plants if seed transmission is confirmed.

3. *Breeding for resistance*
   There is field evidence to suggest either that the cultivars 'SS' and 'Tihore' are resistant to yellow-leaf, or that they act as symptomless carriers. Should either of these cultivars prove to be resistant, a source will be available for the provision of resistant stocks.

Discussion

Previous work failed to reveal a fungus, bacterial or physiological cause for yellow-leaf disease. Although the experiments described in this paper do not eliminate the possibility that *O.atkinsoni* causes yellow-leaf through the production of toxins, this possibility is made unlikely by the fact that hoppers from an apparently yellow-leaf free area did not produce the disease, and because symptoms could be produced by a single hopper from yellow-leaf plants feeding for less than 48 hours.

Transmission of the disease by mechanical means, by grafting, and by *O.atkinsoni* is taken as proof of the virus origin of yellow-leaf disease.

Failure to obtain transmission of the virus to indicator hosts of a number of commonly occurring viruses, together with the endemic nature of the only known vector and its close association with phormium, which is itself endemic to New Zealand and Norfolk Island, makes it highly probable that the virus causing yellow-leaf is endemic to New Zealand.

References

Cottier, W. 1931: The Transmission of Virus Diseases of the Potato by Insects: Leaf-roll. *N. Z. J. Sci. Tech. 13*: 85-95.

Cumber, R. A. 1952: Studies of *Oliarus atkinsoni* Myers (Hem. Cixiidae) Vector of the "Yellow Leaf" Disease of *Phormium tenax* Forst. 1. Habits and Environment, with a Note on Natural Enemies. *N. Z. J. Sci. Tech. B34*: 92-98.